



Newsletter

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From the Editor's Desk:



The past year was a busy one for *INGENIC*. The Second *INGENIC* Workshop took place from November 24th-26th, 1996 in Salvador, Bahia, Brazil. It followed the 12th *International Cocoa Research Conference* (November 21st-23rd). The *INGENIC* Committee is gratified by the growing support for *INGENIC*. This has been demonstrated by the numerous letters received by the Committee, and by the participation of 83 cocoa scientists, representatives of organisations/institutions and interested observers present at the Workshop.

The financial and logistical support for the Workshop from the following institutions is gratefully acknowledged by the *INGENIC* Committee:

ACRI, Almirante Centro de Estudos de Cacau, BCCCA, CEPLAC, CIRAD, CPA, CTA, the Dutch Ministry of Foreign Affairs (DGIS), FAO, IOCCC and the London Commodity Exchange (LCE).

The local organizing Committee for the Workshop comprising of scientists and workers (listed below) from CEPLAC is congratulated for the splendid job done in preparing for the Workshop. The *INGENIC* Committee wishes to express its appreciation to:

Dr. Dário Ahnert (Coordinator)
Dr. Paulo de T. Alvim (Workshop Chairman)
Dr. José Luis Pires
Dr. Joao Luis Pereira
Ms. Ana Eulalia Barbosa M. Almeida

The *Proceedings* of the Workshop are under preparation, and will be distributed in due course.

It is with regret that we announce the resignation of Dr. Rob Lockwood as Vice-Chairman of the *INGENIC* Committee. Dr. Lockwood provided invaluable input in the formation of *INGENIC*, and contributed significantly to the formulation of its objectives and mission. It was he who coined the name for the Group. His presence on the Committee, and at cocoa conferences and gatherings will be greatly missed. However, his sterling contribution to cocoa research will undoubtedly continue to receive recognition, and we wish him success in his present and future undertakings.

We also note with deep sadness the passing of Dr. Simeon Akinwale. The *INGENIC* Committee wishes to extend condolences to his family, friends and colleagues. A tribute to the life of Dr. Akinwale is included in this issue.

This issue contains articles covering a wide range of topics including *Research Priorities for Improvement of Cocoa*, *Genetic Bases of Resistance of Cacao to Black Pod disease*, and a riveting account of a collecting expedition in South America.... The authors are thanked for their valuable contributions. We look forward to future contributions from these and other members of the international cocoa community. Comments on articles previously featured, such as those included in this issue, are welcome.



Frances Bekele



INGENIC News Minutes of the INGENIC Annual General Meeting

25th November, 1996

1. Minutes of previous *INGENIC* General Meeting on 18th October, 1994 were read and confirmed.
2. The Chairman reported briefly on the activities of *INGENIC* since the last GM. These included the compilation and distribution of two Newsletters, preparations for the *INGENIC* Workshop 1997, and the organization of the external reviews on resistance testing and cocoa resistance breeding. Financial and logistical support for these *INGENIC* activities, amounting to more than US \$100,000, had been received from ACRI, Almirante Centro de Estudos de Cacau, BCCCA, CEPLAC, CIRAD, CTA, Dutch Ministry of Foreign Affairs, FAO, IOCCC and LCE. The chairman thanked all those who had contributed to *INGENIC*'s activities.
3. There was some discussion as to whether the *Proceedings* of the *INGENIC* Workshop and the External Reviews should be published together or as separate documents. It was agreed that these documents should be published separately, but that summaries of the external reviews should be included in the *Proceedings*.
4. The Treasurer delivered the financial report and presented an account of the total income and expenditure for 1994-1997. He estimated that *INGENIC* would have a balance of some \$5000 after the publication and distribution of the *Proceedings* for the 1996 Workshop. It was agreed that *INGENIC* should now seek an auditor to examine its financial situation on an annual basis. The Secretary agreed to approach ACRI to see if they would be prepared to act as *INGENIC*'s auditor.
5. The Editor reported that two issues of the *INGENIC* Newsletter had been published since the last GM and distributed to over 250 addresses. She encouraged *INGENIC* members to submit articles or brief notes for future editions.
6. The issue of *INGENIC* membership was discussed, and it was agreed that membership should remain free of charge for the present. The mailing list currently contains 324 addresses. An attempt will be made to update this list to ensure that resources are not wasted. This will be done by attaching an address slip to the next Newsletter.
7. The Chairman announced with regret that Dr. Rob Lockwood has decided to resign as vice-chairman due to a change in his work commitments. The *INGENIC* committee sincerely thanked Rob for having been one of the principal founding members of *INGENIC*, and for all his efforts dedicated to our group and to cocoa breeding in general. The chairman expressed his hope that Rob will be able to continue to give his valuable advice to the committee over the coming years.
8. Dr. Efron read the nominations for the new *INGENIC* committee to which no objections were made. The new members appointed are Dário Ahnert and Yaw Adu-Ampomah as vice-chairmen. Michelle End accepted the roles of Secretary and Treasurer, and Bruce Stillings agreed to act as auditor.
9. The issue of regulations for *INGENIC* was discussed. The Chairman pointed out that the adoption of such regulations may have advantages in handling the financial affairs of *INGENIC* (opening bank accounts, and receiving certain types of institutional support). Some members were in favour of this whereas others had concerns that formal regulations would restrict flexibility. After discussion, it was agreed that the Secretary should obtain examples of simple regulations for societies which could be adapted for use by *INGENIC*. This issue will then be discussed further by the *INGENIC* committee.
10. The Chairman introduced a discussion on forthcoming *INGENIC* activities. It was agreed that the next workshop will coincide with the next International Cocoa Research Conference in Malaysia in 1998 or 1999. Suggested themes for this workshop included "Genotype x Environment Interactions" and "The Use of Modern Technologies in Cocoa Breeding". For the next workshop, emphasis will be placed on presentation of discussion papers rather than research results alone. It was left to the committee to work out details for the next workshop.



Conclusions of the INGENIC Workshop on the *Contribution of Disease Resistance to Cocoa Variety Improvement*

Bahia, Brazil, November, 1996

Economic losses

There is a need to collect more reliable data on real losses incurred due to cocoa diseases and pests. Such information would guide decision makers and researchers in their search for more efficient methods to control these important production constraints.

Phytopathological aspects related to resistance breeding

The response of cacao to its pathogens includes primarily partial, polygenic resistance; it can be measured and exploited by breeders using current techniques including *Components Analysis*.

Early tests for resistance to cocoa diseases have often shown low representativeness, unproven predictiveness and unspecified differential capacity. Nonetheless, the use of such tests, possibly combined with molecular analyses, is imperative to ameliorate progress in resistance breeding.

International consensus on test protocols and on pre-breeding strategies is required, and INGENIC should facilitate discussions leading to such consensus.

Due to the high variability of pathogens, the use of more than one resistance source in the development of new commercial varieties is recommended.

Information on resistance levels observed in the field and in resistance tests should be made available through the *International Cocoa Germplasm Database*.

Resistance to *Phytophthora* pod rot

Resistance evaluation in West Africa, Latin America, the Caribbean and Asia has resulted in the identification of promising clones with increased resistance and/or escape to the disease. In Brazil, some clones were identified with resistance to more than one *Phytophthora* species.

The degree of field attack of individual trees is influenced not only by their intrinsic resistance, but also by the level of attack of neighbouring trees, by pod load and by escape phenomena.

Results from Cameroun, Trinidad and Côte d'Ivoire indicate that the leaf inoculation test is promising for identification of resistance of clones or progenies. Correlations with field resistance were established, and the influence of environment on test results appears weaker with this method than with field inoculation of pods.

Ranking of the general combining ability of a number of genotypes for field disease incidence in Cameroun, Togo and Côte d'Ivoire suggests that field reactions are consistent between countries, despite the different *Phytophthora* species involved. This finding is in line with absence of interaction between fungal isolates and cacao genotypes observed in leaf inoculation tests.

In Trinidad, a rapid resistance evaluation method was developed involving spray inoculation of detached pods. This test seems promising for application in a pre-breeding programme, such as that already initiated by CRU.

Test methods differ between countries. More data are being collected in laboratories than in the field and should be compared with field results. Standardization is important to confirm the representativeness of different resistance tests and their use in international collaborative studies.

Results from Costa Rica and Côte d'Ivoire indicate that significant links between resistance to Black Pod and molecular markers can be established. QTL studies are being continued in collaborative research programmes.

Resistance to Witches' Broom disease

Resistance reactions, evaluated by broom growth, broom death and basidiocarp production can be used to identify useful selections in collections. Isolates differed greatly in aggressiveness, but ranking order remained the same indicating that resistance against one fungal isolate might also be useful in relation to other isolates. It was recommended that work related to challenging of promising cacao genotypes with different fungal isolates be continued outside cocoa-producing countries.

The Workshop stressed the need for further studies on the reliability of early screening tests for Witches' Broom disease that are adapted to the breeders' needs of rapid evaluation of clones and progenies. Screening for resistance using cacao seedlings, as practised in Brazil and Ecuador, has shown a good correlation with field resistance and permitted the identification of promising new materials. Further work on the leaf inoculation test, under development at CRU, Trinidad, as well as the sap germination test, under development at CEPLAC, Belém, is to be encouraged.

Resistance evaluations of collections in Brazil revealed higher resistance in Upper Amazon material than in Lower Amazon material. Other sources of resistance than the well-known Scavina clones were identified in collections in Brazil as well as other countries (Peru, Trinidad, Ecuador). Furthermore, more resistant material was identified in Ecuador in a national survey carried out in farmers' fields. These reports suggest that breeders can rely on several different sources of resistance to this disease.

International collaboration is needed to exchange different sources of resistance, so as to facilitate the accumulation of resistance genes.

Resistance to Vascular Streak Dieback (VSD)

The *in vitro* dual culture laboratory method was found reliable and could be used for rapid screening of clones or individual trees within progenies as well as for research into the host-pathogen interaction, and their application by cocoa breeders.

Inheritance studies indicated that resistance to VSD is heritable, polygenic and additive. Accumulation of VSD resistance genes is emphasised, together with resistance to *Phytophthora* pod rot.

International collaboration is required for exchange of material, standardization of resistance test methods and their application in breeding programmes.

Other diseases

Exchange of clones with resistance to *Monilia* is recommended along with realisation of tests under field conditions. Recent evaluation of collections in Peru indicates the great severity of this disease, but variation in disease incidence between accessions was observed.

Mutation breeding appears to have promise in creating material with resistance to Cocoa Swollen Shoot Virus (CSSV) as well as with variation for other useful traits.

Resistance to *Ceratocystis* was identified in Venezuela, and this has been incorporated into commercial varieties.

Breeding

Advances in cocoa breeding are best achieved by (reciprocal) recurrent selection schemes, such as those adopted recently in a number of countries. These methods can take advantage of the predominantly additive gene action observed for most selection traits in cacao, and will increase chances of detecting transgressive types. Resistance breeding is to be integrated into such programmes that should simultaneously take into consideration other selection criteria such as yield, bean size, uniform plant type and quality.

Quick progress can be obtained by fixation of interesting genotypes by clonal selection. Improved methods of vegetative propagation, including micropropagation, therefore have a role to play in the rapid distribution of clones resistant to destructive cocoa diseases.

Recombination of resistance genes of different origins is expected to allow quick selection progress as it is likely that resistance genes with relatively large effects on partial resistance can be found and efficiently accumulated.

Conclusion

Good field data obtained under local conditions are the best basis for the start of any breeding effort for increasing resistance, but pre-selection tests are essential in obtaining satisfactory progress.

Consideration should be given to re-designing the cacao tree architecture in order to not only improve photosynthetic efficiency and harvest index, but, concurrently, to increase the effectiveness of available partial resistance to cocoa diseases.

Such programmes need breeders who combine expertise in genetics with general knowledge in agronomy and crop protection. In addition, effective team work involving pathologists, entomologists etc., competent field staff for excellent field trial execution, and last but not least, adequate funding are essential.

Global projects related to germplasm utilization and conservation deserve international support from producer and consumer agencies. Any pre-breeding programme at a germplasm centre should be endorsed by national cocoa breeders, who are in the best position to decide on the selection criteria and material to be used in such a programme.



Comments from HilleToxopeus

"I submit the following Summary of some thoughts as a reaction to the "Conclusions and Recommendations from the IWCBS...." as on p. 3 of *INGENIC Newsletter Issue 1*."

H. Toxopeus

What about populations?

- The word *POPULATION* is conspicuously absent from the text.
 - Do *populations* not feature anymore in cocoa breeding?? It would seem that they are considered inconsequential and therefore not in need of Research and Development (R&D) support.
 - "Mendelian" populations (of trees) are the physical basis of *Theobroma cacao* L.. They are most effective containers of genetic variation, as in all random mating, seed reproduced, cultivated plant species.
- A-
- This population concept implies that heterosis and inbreeding, including the combining abilities, are part of cocoa genetics.
- A1 - That these phenomena occur is confirmed by cases of heterosis with evidence both direct and circumstantial, as summarized in my paper for the first conference on "Cocoa and Coconuts in Malaysia" in Kuala Lumpur in 1971 ("*Cocoa Breeding, a consequence of mating system, heterosis and population structure*"). The occurrence of inbreeding (point 3 of the **Conclusions**; What is the evidence?) is additional proof. That the various combining abilities have been shown to occur completes the picture.
- A2 - Cocoa breeding, as from the 1950's, identified and successfully exploited "combining ability" particularly with regard to juvenile vigour in hybrid (vigour) varieties consisting of mixtures of hybrids mostly. This combining ability was found to express itself in crosses between populations. The strategy of "wide crossing", i.e. crossing clones from Upper Amazon (UA) parentage with local (identified or not) selections, and extensive field testing, produced the hardy and apparently sustainable varieties of the West African cocoa belts. They apparently produce the right type of beans because differences of quality are between countries, i.e. are the result of differences in quality control systems.
- The vigour causes plants to grow rapidly through field establishment, to yield early and close canopy quickly. This is their great advantage over West African Amelonado at least in West Africa; whether they really yield more than West African Amelonado (WAA) when mature is questionable.
- There is the somewhat reductionist aspect of a narrow genetic base, however, the varieties were the end product of an elaborate selection process from a much broader genetically diverse gene pool.

A3 - Sure enough the varieties are lacking in disease resistance. The experience in developing resistance is largely negative, but for the shiny example of the Witches' Broom disease resistance genes built into the Pound/Freeman variety of plant material in Trinidad's cocoa, which, after roughly 25 years is still fully resistant; a case of durable resistance! Breeding for resistance requires a more elaborate pre-breeding phase. As stated in my article in **Issue 3** of the *CRU Newsletter*, a population improvement approach is advocated. I worked out the case in more detail in "*Breeding for Black Pod Resistance in T. cacao L.*" in "*Phytophthora Disease of Cocoa*" (1974) by Prof. P.H. Gregory. It stresses that apart from screening for resistance using well-tested screening methods, populations should be improved in local adaptation and commercial qualities, obviously considering genetic limitations. The key term here is :

POPULATION IMPROVEMENT and it should be added to RECURRENT SELECTION as mentioned in the "Conclusions...." *INGENIC Newsletter Issue No. 1*.

B - Cocoa genetic resources are contained in populations and they are the physical basis of the hybrid breeding strategy.

B1 - An overview of the populations of *T. cacao* is given in my paper as above, and in "*Botany, types and populations*", chapter 2 in "*Cocoa*" (1985).

B2 - A peculiar advantage of populations is that their genes and alleles, and cytoplasm may be transferred as packages of seeds. Applying the rules properly, a couple of hundred fresh cocoa seeds weighing roughly 1 kg will contain the whole genetic variation of a population.

B3 - Managing the genetic variation of small populations revolves around the concept of "effective population size" (Allard, *Principles of Plant Breeding* (1960), p 200-203: "*Random Mating in Small Populations*"). This is determined by the number of parents physically involved in seed reproduction: the "effective" parents. The number of effective parents is given by the formula $4N_f N_p / (N_f + N_p)$, N_f being the number of female parents and N_p the pollinators. For example, in the case of 25 trees of a population each being effectively hand-pollinated by pollen from each of 25 other trees, the number of effective parents is 50. See also my statement on collecting populations in the third issue of the *CRU Newsletter* (1996).

The gene loss incurred by moving to a next generation expressed as the loss of heterozygous loci is estimated by the formula $1/2N$, N being the number of effective parents. The outcome of our example: $1/100 = 1\%$ is the proportion of heterozygous loci that will become fixed (homozygous) in the next generation: 1 out of 100, small indeed. (ref. Allard 1960, p. 200).

B4 - The 25 pods realised in the example will contain an average of 750 seeds that would result in about 700 plants: a good start for a recurrent selection and improvement programme. I believe that effective population size should be kept to about 50 parents in the first

few generations of selection so that several selection goals (general adaptation, disease resistance and pod and bean values) may be pursued effectively. The selection pressures applied will thus result in shifts of gene frequencies towards the desirable genes rather than undue fixation and the loss of other potentially useful alleles (genetic drift). Deliberate fixation of loci with useful genes may subsequently be realized by reducing the number of effective parents to perhaps 5 superior trees.

Developing a concrete plan of action with an international dimension, including logistics, timing and costing would take considerable thinking and discussion, a worthwhile subject for a substantial workshop.

C - So as to provide for the most effective transfer of genes, at the time of planning the International Cocoa Genebank, Trinidad (ICG,T) (around 1985), it was decided to replant Pound's 1938 introductions by population so as to facilitate the reconstitution and reproduction of each population by hand or open pollination.

C1 - I suspect that the "genepool of Parinaris" mentioned in the *CRU Newsletter Issue 2* on page 7 is in fact the reconstitution of the PA population. This is good news for population improvement; hopefully the other populations follow soon. This effort should be strengthened!

C2 - In 1989, at the request of FAO, Dr. Kennedy and I developed an outline of a case of population improvement based on the ICG,T populations in a paper: "*Effective use of cocoa genetic resources as exemplified by the International Cocoa Genebank Trinidad*" of FAO Plant Production and Protection Paper No. 94: "*Effective Utilization of Germplasm*" (1989), p.59-81.

D - My conclusion is that the various loose ends of population improvement should be tied up, worked out and costed in time and money, without further ado.



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Future Developments, Research Priorities, and Policy for the Improvement of Cocoa: Results of a Delphi Expert Investigation

Nikolaus Gotsch

Introduction

Biotechnology may support the agricultural sector in developing countries; in particular, the direct use of biotechnologies for plant breeding could dramatically raise crop productivity and overall food production in developing countries. The reverse side, however, is that these technologies may jeopardise developing countries' exports directly through substitution or through a decrease in international commodity prices owing to enhanced physical output. As an example for an agricultural export commodity important for developing countries the author assesses promising biotechnical developments for cocoa, and the socio-economic effects of their adoption on producer countries and the world market within the scope of a three-year post-doctoral research project. In a first step, a written repeated expert investigation (Delphi survey) was accomplished. It evaluates and discusses the research status and future developments in cocoa biotechnology. The survey consisted of three rounds. Round one gathered information on cocoa research activities, prospects and constraints and did not include quantitative forecasts. In the second and third round, it was the task of the experts to assess quantitatively the chances of specific techniques and technological developments. The exact course of the future development of a technology depends on external social, political, legal, and economic conditions, which may vary considerably for long forecasting periods. For this purpose, three different environments- so-called scenarios- have been formulated and served as a basis of the experts' forecasts. The questionnaire of the first round was sent in December 1994 to 92 experts from 23 countries. Of the 29 questionnaires submitted in the third round, those of 27 experts from 14 countries were taken into account for the final analysis.

Main findings

Based on the results of the third round, the following promising developments may be achieved through a combination of various research tools within the next 25 years (approximately):

- Phenotypic characterization of accessions in cacao genebanks will support the identification of associated characteristics and facilitate the detection of mislabelled accessions and duplicates and thus make traditional breeding more efficient. This could make available to breeders thousands of uncharacterized accessions in genebanks.
- Molecular markers linked to disease resistance loci will provide essential information to breeders. The experts agree with the argument that improved genetic knowledge of breeding materials will ensure the development of improved varieties.

- Fingerprinting of pathogens can give useful indications of the genetic make-up of the pathogen and the species of the pathogen (for instance different *Phytophthora* species). Thus, in any cocoa-growing region, it would be beneficial to know the genetic make-up of the pathogen population one is breeding against. Furthermore, information on the genetic make-up of pathogens could provide important information for ecological approaches to biological control and integrated control.
- Somatic embryogenesis and the ability to induce somatic embryos to develop into rooted plantlets may allow more efficient propagation and multiplication of superior clones, and more efficient conservation and exchange of valuable breeding material.
- The experts expect new cultivars with an improved yield potential and with improved cocoa butter content and fatty acid profile.
- New cultivars with improved resistance to important diseases and pests of cocoa may become available to cocoa production within the next 25 years, for instance to *Phytophthora* pod rot, vascular streak dieback, Witches' Broom disease, and the cocoa pod borer.

In the course of the various survey rounds, a lively controversy developed with respect to the importance and interdependence of various research tools, in particular concerning the role of modern biotechnology (*in vitro* methods, molecular biology, and genetic engineering) and its integration into traditional applied R&D, especially practical breeding. The experts' quantitative assessments demonstrate that a worsened research environment would cause the collapse of research progress in cocoa, whereas a more advantageous institutional environment and ameliorated research funding would considerably improve future research progress. It becomes evident from the survey that there is strong competition for financial resources in cocoa research and development. New methods of molecular biology and biotechnology compete for scarce, and in many cases shrinking funds for the traditional methods of applied R&D. A serious threat to traditional fields of R&D, in particular cocoa breeding, is expressed by various experts active in these fields, in particular by breeders. The development of micropropagation methods are expected to increase the work of traditional plant breeding programmes because only breeders would be able to effectively test the new varieties emerging from laboratories. Producer countries must therefore strengthen their capacity for applied R&D. This, of course, requires additional funding. In fact, traditional breeders fear that they will not be able to meet these additional requirements in a situation where even up to now the level and continuity of funding and personnel resources devoted to cocoa breeding have rarely been commensurate with the task.

Those experts expecting good prospects do not deny that perennial plants will still be perennial plants. The usefulness of novel characteristics will always need to be established in the field. Breeding progress will continue to be a major tool in applied R&D in cocoa for the foreseeable future. The need for an improvement of resistance/tolerance characteristics to diseases and pests is agreed by all parties involved in cocoa research. Besides the ecological advantages of improved resistance to pathogens -

allowing a reduction of ecologically harmful pesticides - these cost-reducing biotechnological inputs would permit effective competition at reduced world market prices in view of the saturated world markets. Therefore, an assessment of the expected socio-economic impact of promising future technological developments in cocoa has to be carefully analysed and should be in the interest of all producer countries. For this purpose, the author is developing an economic model which assesses the future effects of the adoption of promising biotechnological developments for cocoa on producer countries and the world market.

Existing publications on cocoa R&D primarily present the research status and production constraints for this crop, but give only a limited perspective concerning the potential for future developments. The results of the study presented here may provide information to policy decision makers in the process of priority setting of the future cocoa R&D policy. Furthermore, this study may support cocoa producers and researchers in choosing future production strategies.

I would like to thank the experts who spent considerable time and thought in order to complete the various survey rounds providing the necessary technological information to social scientists for a sound socio-economic assessment of the impacts of future biotechnical developments in cocoa.

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High Efficiency Somatic Embryogenesis and Genetic Transformation of Cacao

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Siela Maximova and Sharon Pishak

Cacao trees (*Theobroma cacao* L.) exhibit a high degree of genetic heterozygosity primarily because of the common occurrence of reproductive self-incompatibility. In a typical cacao planting that contains a large population of trees grown from genetically different seeds, relatively few trees produce exceptional yields, and about one third of the trees produce below average yields. Hence, there is a desire to vegetatively propagate the higher-yielding trees. Additionally, recent advances in breeding/selection programmes have identified clones exhibiting superior disease resistance traits which can segregate in seed derived progeny. During the past thirty years, the vegetative cloning of superior genotypes or selected trees produced through breeding programmes has been proposed as a means to increase the overall yield, quality and agronomic performance of cacao. However, in spite of a great deal of effort to devise improved methods for vegetative propagation, cacao trees are currently commercially reproduced only via seeds and cuttings. Somatic embryogenesis is the process by which somatic cells undergo bipolar development to give rise to genetically identical whole plants by means of the development of adventitious embryos that occur without the fusion of gametes. The recent development of somatic embryogenesis systems for cacao has opened a new avenue for vegetative propagation, but the published methods are not highly efficient and are sensitive to genotypic variability.

Additionally, like many of the major domesticated crop plants, the majority of cacao commercially cultivated today is derived from a few varieties collected 50-60 years ago, thus having a relatively narrow genetic base. In part due to this fact, cacao remains extremely vulnerable to diseases and other abiotic stresses. Up to 40% of the world cacao crop production is lost each year due to fungal and viral diseases and to attack by various insect pests (H.A.M. van der Vossen, Report to INGENIC, 1996). Continued improvement in cacao production, through the development and utilization of superior genotypes with desirable yield and bean quality characteristics, resistance to diseases and insect pests, and tolerance to drought and cold, via conventional and modern breeding methods is the major objective of the INGENIC organization. As an additional tool for the introduction of genes into cacao germplasm, genetic transformation holds a powerful promise for the future. This ability, which has now been extensively utilized for many major crop species, allows the transfer of genetic material between virtually any organisms, vastly increasing the potential genetic base which is utilized for crop improvement. However to date, no method for the genetic transformation of cacao plants has been reported.

We have recently developed improved protocols for somatic embryogenesis in cacao, and have achieved the production of transgenic cacao embryos using non-tumorigenic strains of *Agrobacterium tumefaciens*. Furthermore, we have developed methods for conversion of such embryos into plantlets, and for the acclimatisation and transfer of these plantlets into the greenhouse. Combining these techniques, we have produced for the first time, transgenic cacao plantlets. These methods open many possibilities for improvement of cacao germplasm and for the

multiplication of superior genotypes. Genes from virtually any organisms can now be moved into cacao. We describe here the general features of our methods; detailed manuscripts are in preparation and will be published elsewhere.

Somatic Embryogenesis

The embryogenesis system developed in our laboratory involves the use of several culture steps in combination with the use of the synthetic cytokinin thidiazuron (TDZ) and a synthetic auxin (2,4-D). These steps include: callus induction, embryo development, and plant regeneration processes. In the first step, rapidly growing compact callus is induced from floral explant tissues, including staminodes (needle-like tissue fused with filament at the base of stamen) and petal base structures, on a callus induction medium composed of DKW basal medium (Driver and Kuniyuki (1984) *Hortscience* 19 507-509) supplemented with TDZ and 2,4-D. Callus tissue is subcultured onto a callus maintenance medium containing WPM basal medium (Lloyd and McCown (1981) *Int. Plant Prog. Soc. Proc.* 30 421-427) supplemented with a reduced concentration of cytokinin and 2,4-D, to further stimulate bipolar callus cell development and embryo differentiation. During the callus culture steps, glucose is used as the sole carbon source in all the culture media in order to achieve maximum growth rates. Mature somatic embryos are obtained through transfer of embryogenic callus onto an embryo development medium containing DKW basal medium, and sucrose. Germination of somatic embryos and regeneration of whole plantlets is achieved by transfer of somatic embryos to a germination medium consisting of a diluted DKW basal medium, containing a combination of glucose and sucrose.

Using the above described procedure, high frequencies of somatic embryo production and plant regeneration from cultured floral explants can be readily obtained. For example, up to 100% and over 60% of cultured staminode and petal base explants, respectively, from cacao genotype Sca 6 produced somatic embryos. A single staminode explant produced up to 140, and an average of 46 primary somatic embryos. In addition, efficient repetitive embryogenesis was also obtained. We have observed that a single primary embryo produced more than 50 secondary embryos after being subjected to an extended culture period of one month. Theoretically, this results in a potential of up to 7,000 embryos from a single staminode! The procedure was also, in part, genotype-independent. Thus far, all 19 cacao genotypes tested have produced somatic embryos, however the efficiency of embryo production does vary between genotypes with between 46% and 1% of staminodes producing embryos. Up to 90% of the selected mature somatic embryos that are produced using this procedure were capable of conversion into plantlets. After transfer into the greenhouse, nearly all plantlets exhibit normal growth and morphology compared to seed grown plants.

The somatic embryogenesis procedure we have developed is greatly simplified in comparison with previously described methods. The length of time required for the production of somatic embryos and plantlets, the labour, and thus the cost involved, are greatly reduced. More significantly, the efficiency of somatic embryo production and plant regeneration from staminode and petal base explants is dramatically improved, and while genotypic variation is still observed, all genotypes tested thus far have produced embryos. In total, these improvements allow for the practical use of somatic embryogenesis for cacao clonal propagation and other applications that require the production of large quantities of plants from limited source materials. To date, a

detailed field performance trial of somatic embryo derived plants has not been conducted. However, preliminary field trials of somatic embryo derived plants by several groups have been encouraging. Until such a trial is completed, including yield and quality measurements, use of plants produced by this method must be done with caution.

Genetic Transformation

Cells of *Agrobacterium tumefaciens* strain EHA101 harbouring a disarmed version of the agropine-type supervirulent Ti plasmid pTiBo542 (Hood *et al.* (1986) *J Bacteriol* 168 1291-1301) and a binary plasmid were induced to increase their infectivity through a series of treatments including; culture in low-pH induction medium and preconditioning with wounded tobacco leaf extract in order to induce *Agrobacterium* vir gene expression. Immature somatic embryo tissues derived from either staminode and/or petal base explants of young cacao flower buds, are co-cultivated with the incited *Agrobacterium*. Selection for transformed callus tissue is achieved by culturing embryo tissues in liquid culture medium containing selective antibiotics. Transformed callus masses that are subsequently identified are isolated from the parental embryo tissue based on the expression of the green fluorescent protein (GFP) gene which had been incorporated in the T-DNA region of the binary plasmid. The fluorescence of this reporter gene product can be monitored non-destructively using a epi-fluorescence stereo microscope. Transgenic somatic embryos are induced from the recovered transformed calli and converted into plantlets using procedures previously defined in our laboratory.

A number of GFP positive callus colonies have been obtained, and from some of these colonies, a large number of somatic embryos have developed. The expression of the GFP gene in the transgenic embryos has been stable throughout all developmental stages, as determined by the presence of the GFP-specific green fluorescence. Mature transgenic embryos have produced roots and are being converted into plantlets on a modified germination medium.

Successful transformation of cacao cells, and the subsequent production of transgenic somatic embryos and plants using the *Agrobacterium*-mediated transformation procedures defined in this laboratory, provide a new tool for the introduction of foreign genes into cacao, and an alternative approach for the incorporation of novel mechanisms of resistance to viruses, fungi and insect pests. In addition, this technology may enable the development of transgenic cacao varieties with improved agronomic performance characteristics, and also be useful in providing a new experimental system for study of pathogenesis and of gene expression and function, in cacao. Researchers interested in using these systems are encouraged to contact our group for discussions of technology transfer and/or collaborative research.

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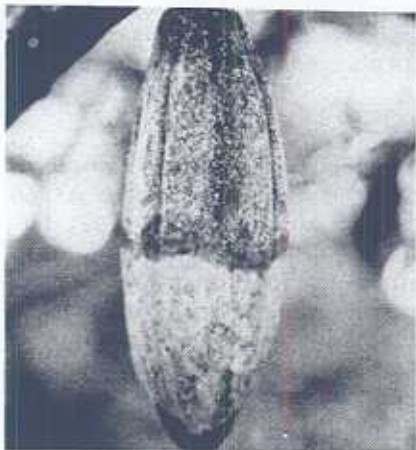
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An International Project on the Genetic Bases of Resistance of Cacao to *Phytophthora*

Denis Despréaux and Albertus Eskes

Phytophthora spp. diseases, particularly black pod, are a major problem of cocoa cultivation. Crop losses worldwide are estimated at 15% but they can be much higher in some areas, such as in central Africa, where the destructive *P. megakarya* species causes losses up to 80%. Chemical control methods have been developed which, combined with frequent phytosanitary harvests, may help to considerably reduce the disease. Little progress has yet been made on genetic resistance and it is not yet possible to integrate this character when implementing an integrated control scheme. However, earlier work has shown that there is considerable variation in the reactions of the different genotypes to the disease, though complete resistance has not been found. The resistance phenomena observed have also proved to be heritable.



Black Pod disease of cocoa (Photo: T.N. Sreenivasan)

An international project was initiated in July 1995 with the objective to increase our knowledge of the genetic bases of resistance to *P. palmivora* and *P. megakarya*. The following research activities are being undertaken:

- (1) phenotypic characterization of resistance factors and comparison of different evaluation methods;
- (2) identification of quantitative trait loci (QTL) based on molecular markers (isozymes, RFLP, AFLP, microsatellites) and locating these QTL on a genetic map comprising more than 350 markers; and
- (3) initiation of accumulation of resistance factors in crosses between resistant parents. The research is based on individual tree records of progenies in existing field trials in Cameroun (*P. megakarya*) and Côte d'Ivoire (*P. palmivora*) as well as on new progenies to be created in these countries and in Trinidad (*P. palmivora*), which will be evaluated by early screening methods. Four research organizations are implementing the project in partnership: IRAD (Cameroun),

IDEFOR (Côte d'Ivoire), the Cocoa Research Unit (Trinidad) and CIRAD (France), which is acting as coordinator. The project receives substantial financial support from CAOBISCO (European Association of Chocolate, Biscuit and Confectionery Industries) and has a duration of five years.

During the first 18 months of the project, activities have included the evaluation of resistance of parental clones and individual trees of several progenies, mapping with genetic markers of four progenies and creation of crosses between selected genotypes. Resistance is assessed in the field by the level of attack of individual trees observed over many years, as well as by artificial inoculation of pods and leaves carried out in the field or laboratory. Correlations between these traits were satisfactory when the means of progenies or clones were concerned. Based on individual tree data, heritabilities for these three characters appeared also to be high but resistance of leaves and pods appeared to be unrelated and showed low correlations with the field level of disease. The reason for this is under investigation and the possible influences of other factors (pod traits, effect of the period of pod production, environmental aspects) are taken into account.

Regarding QTL identification, the first population analysed involved 144 trees located at Bingerville, Côte d'Ivoire, belonging to a cross between two heterozygous parents: UPA402, an Upper Amazon Forastero and UF676, a Trinitario. A saturated genetic linkage map was constructed using molecular markers. Resistance traits were evaluated using a leaf disk test and four years of field data. Two QTL's for field resistance were identified for each of the parents, explaining 46% of total variation. These findings confirm earlier results obtained on a smaller number of trees of the same progeny (N'Goran *et al.* (1995), in: *Proc. Int. Workshop on Cocoa Breeding Strategies*, Kuala Lumpur, Malaysia, *INGENIC*, 1995) and suggest that molecular markers could be used for "marker assisted selection". With regard to the resistance of leaves, the results were less conclusive although two regions in the genome were identified that appear to be related both to field and leaf resistance.

With regard to accumulation of resistance factors, several crosses between selected parents of different origin were created in Trinidad. Results obtained with the pod and leaf resistance tests applied to a large number of potentially interesting parental clones were satisfactorily correlated. Presently, the progenies growing in the nursery are being evaluated for leaf resistance.

These promising early results are being completed to permit further accurate evaluation of the resistance factors involved to fully assess the potential of marker-assisted selection, and to accumulate different resistance factors in new cacao varieties.

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Figure 1: Dendrogram based on Cluster Analysis (UPGMA Method) of the 28 cacao populations under study.

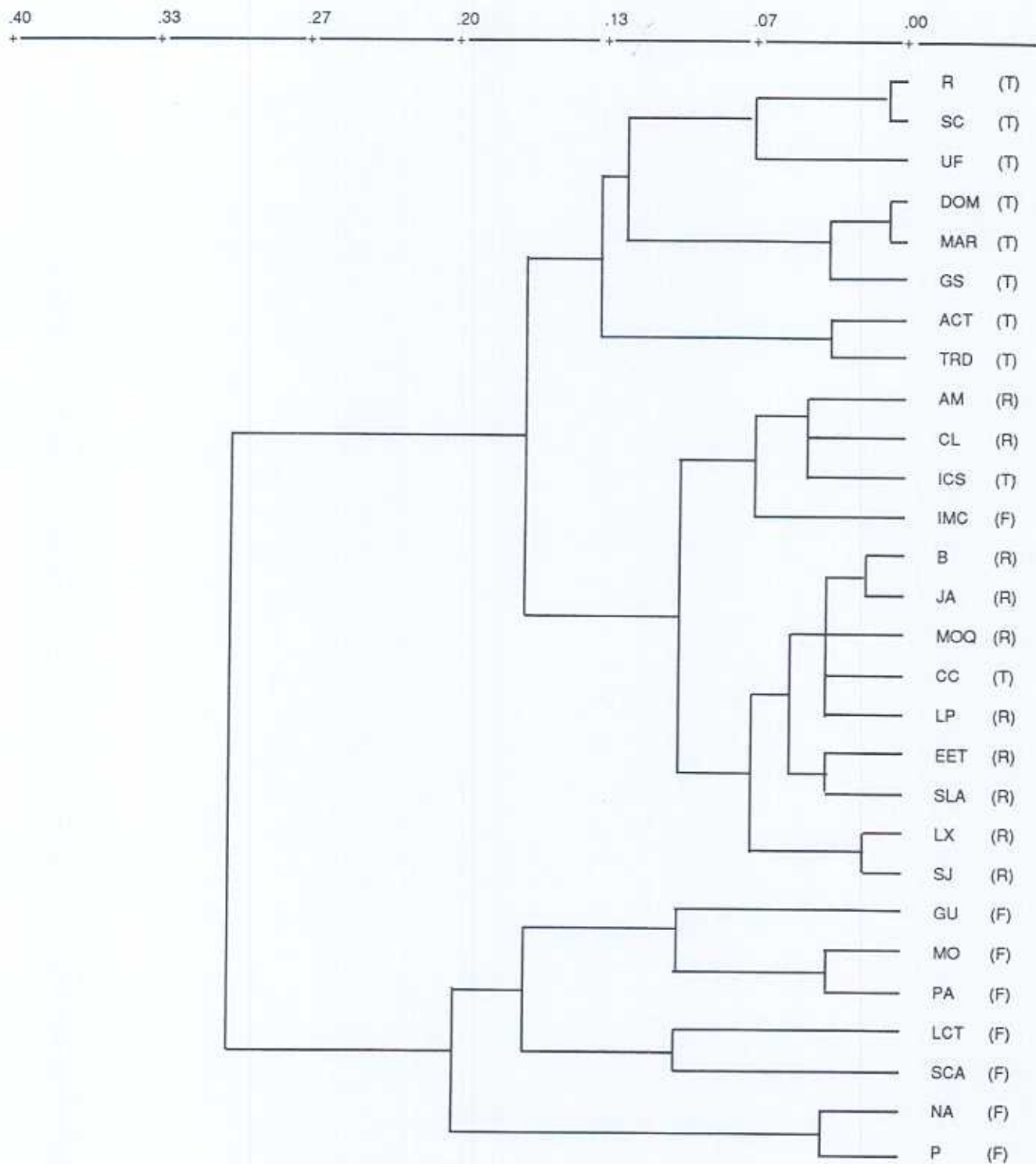


Table 3: Diversity of the different "populations", estimated by several indices calculated from I.E data. (The numbers in parentheses represent the standard errors).

Population	% polymorphic loci	% heterozygosity	Mean number of alleles per locus	Shannon diversity index	Nei diversity index
ACT	66.7	23 (13)	1.8 (0.3)	0.5	0.29 (0.11)
AM	83.3	40 (10)	2.2 (0.3)	0.84	0.40 (0.11)
B	83.3	36 (11)	2.3 (0.3)	0.88	0.41 (0.1)
CC	83.3	46 (16)	2.2 (0.3)	0.6	0.40 (0.11)
CL	83.3	33 (11)	2.5 (0.4)	0.92	0.39 (0.1)
DOM	66.7	16 (6)	1.8 (0.3)	0.38	0.16 (0.06)
EET	83.3	43 (13)	2.3 (0.4)	0.87	0.41 (0.11)
GS	66.7	30 (10)	2.0 (0.3)	0.58	0.26 (0.09)
GU	33.3	13 (9)	1.5 (0.3)	0.34	0.14 (0.1)
ICS	83.3	42 (11)	2.5 (0.3)	0.91	0.41 (0.08)
IMC	66.7	31 (10)	2.3 (0.4)	0.6	0.27 (0.08)
JA	83.3	35 (11)	2.3 (0.4)	0.93	0.40 (0.1)
LCTEEN	100	29 (6)	2.7 (0.3)	1.04	0.44 (0.07)
LP	66.7	35 (12)	2.0 (0.4)	0.73	0.34 (0.12)
LX	66.7	37 (14)	2.0 (0.4)	0.61	0.35 (0.11)
MAR	33.3	6 (4)	1.3 (0.2)	0.16	0.06 (0.04)
MO	83.3	31 (10)	2.2 (0.3)	0.71	0.36 (0.11)
MOQ	83.3	44 (13)	2.3 (0.4)	0.89	0.42 (0.11)
NA	83.3	19 (8)	2.3 (0.2)	0.74	0.33 (0.07)
P	83.3	18 (10)	2.3 (0.3)	0.55	0.27 (0.05)
PA	83.3	18 (6)	2.0 (0.3)	0.67	0.29 (0.08)
R	83.3	67 (21)	1.8 (0.2)	0.08	0.40 (0.09)
SC	83.3	66 (18)	2.2 (0.3)	0.38	0.44 (0.09)
SCA	83.3	30 (9)	2.2 (0.3)	0.79	0.36 (0.1)
SJ	66.7	41 (13)	1.8 (0.3)	0.62	0.35 (0.11)
SLA	83.3	26 (10)	2.3 (0.4)	0.84	0.43 (0.1)
TRD	83.3	22 (8)	2.0 (0.3)	0.60	0.29 (0.07)
UF	100	50 (12)	2.5 (0.3)	0.77	0.43 (0.06)

Table 2: Accessions studied by Isozyme Electrophoresis.

POPULATION	ACCESSIONS STUDIED
ACT	2-13, 2-14, 2-18, 2-19, 2-6, 2-8, 3-2
AM	1107, 128, 13, 15-3, 157, 187, 221, 238, 253, 262, 212
B	13-5, 137, 14-9, 185, 2-16, 2-34, 202, 22-3, 236, 5-7, 53, 7.38, 721, 73, 738, 85, 17-17
CC	71, 9, 38, 39, 34, 41, 54, 49
CL	10-11, 10.10, 10.33, 1026, 13-12, 13-17, 13-2, 13-65, 13.36, 134, 19-2, 19.21, 19.41, 1922, 27-109, 27-49, 2753, 916, 917, 951
DOM	1, 15, 16, 21, 24, 25, 3, 35, 4, 27, 31
EET	156, 19, 338, 397, 399, 400, 401, 59, 95, 162
GS	10, 12, 17, 26, 29, 4, 48, 50, 58, 61, 62, 71, 77, 78, 46
GU	151, 219, 241, 243, 255, 261, 265, 271, 286, 300, 305, 307, 310, 322, 114, 351
ICS	1,100, 14, 16, 17, 2, 26, 28, 29, 30, 40, 42, 43, 48, 49, 53, 55, 56, 57, 6, 61, 62, 65, 67, 73, 75, 76, 82, 83, 84, 99, 39
IMC	10, 103, 107, 12, 14, 16, 2, 3, 30, 36, 39, 45, 47, 49, 57, 59, 60, 61, 65, 67, 73, 77, 96, 97
JA	1-4, 10-35, 10-41, 10-42, 1052, 116, 12, 2.18, 224, 3-2, 3-30, 3.7, 314, 322, 5-2, 5-27, 5-7, 519, 923, 937, 528
LCTEEN	62/1010, 201, 23, 246, 250, 28/S1, 302, 31, 326, 46, 62/S4, 67, 73A, 83/S8, 85, 163
LP	1-10, 1-49, 143, 158, 161, 19, 213, 214, 313, 329, 335, 4.7, 41, 412, 44, 443, 45, 5.1, 519
LX	1, 16, 17, 18, 28, 32, 39, 44, 45, 53, 6, 31
MAR	1, 10, 11, 12, 13, 14, 19, 21, 22, 9, 3
MO	125, 28, 3, 4, 81, 83, 9, 90
MOQ	11, 3.8, 5.102, 523, 529, 55, 6.102, 636, 672, 619, 630, 667, 699, 6.104
NA	1, 12, 13, 149, 154, 178, 186, 191, 218, 229, 244, 258, 286, 3, 311, 34, 342, 387, 406, 45, 471, 528, 685, 7-10, 7-11, 708, 716, 730, 750, 766, 770, 780, 84, 876, 935, 95, 90
P	10B, 10C, 15A, 16B, 189, 18A, 1B, 25A, 25C, 26C, 27C, 2A, 4A
PA	107, 118, 121, 135, 141, 169, 188, 191, 189, 194, 200, 218, 3, 30, 4, 45, 46, 61, 70, 72, 105
R	10, 101, 113, 19, 2, 24, 39, 41, 48, 106
SC	1, 13, 15, 17, 19, 20, 3, 4, 6
SCA	12, 19, 23, 24, 3, 5, 6, 8, 9
SJ	1.39, 118, 119, 12, 137, 140, 219, 22, 222, 226
SLA	13, 16, 20, 53, 54, 67, 66, 8, 93, 95
TRD	1, 118, 16, 45, 46, 5, 53, 81, 9, 92, 6, 13, 41
UF	12, 122, 29, 38, 4, 602, 613, 667, 700, 705, 709, 668

Table 1: Characteristics of the "populations" under study.

Population	Origin	Group	Sample size
ACT	Trinidad	mixed	7
AM	Ecuador	Refractario	11
B	Ecuador	Refractario	17
CC	Costa Rica	Trinitario	8
CL/CLM	Ecuador	Refractario	20
DOM	Dominica	Trinitario	11
EET	Ecuador	mixed	10
GS	Grenada	Trinitario	15
GU	French Guyana	Forastero	16
ICS	Trinidad	Trinitario	32
IMC	Peru	Forastero	24
JA	Ecuador	Refractario	21
LCTEEN	Ecuador	Forastero	16
LP	Ecuador	Refractario	18
LX	Ecuador	Refractario	12
MAR	Martinique	Trinitario	11
MO	Peru	Forastero	8
MOQ	Ecuador	Refractario	14
NA	Peru	Forastero	37
P	Peru	Forastero	13
PA	Peru	Forastero	21
R	Mexico	Trinitario	10
SC	Venezuela /Colombia	Trinitario	9
SCA	Peru?	Forastero	9
SJ	Ecuador	Refractario	10
SLA	Ecuador	Refractario	10
TRD	Trinidad	Trinitario	16
UF	Costa-Rica	Trinitario	12

Indeed, some "populations" show very limited diversity in terms of allelic richness as well as heterozygosity, such as: **GU**, **MAR** and **DOM**. Conversely, other populations show a high level of diversity for both allelic richness and heterozygosity, such as **UF**, **MOQ**, **ICS**, **EET** and **AM**. Some other populations show a high level of heterozygosity, but a low level of genotypic diversity or of allelic richness, as in the cases of **R** and **SC** populations. Others such as **LCTEEN** and **SLA** show a low level of heterozygosity and a high level of allelic richness.

In some cases, these differences reflect the various ways in which these "populations" were obtained. For example, the **LCTEEN** "population", which is in reality a group of populations, has been collected over a very large area (roughly 160.000 km² (Allen and Lass, 1983)), resulting in a high level of diversity in the sample present in the ICGT. On the other hand, the accessions from the **GU** population, which we have analysed, have been collected in a much more restricted area (roughly 80 km² (Lachenaud and Sallée, 1993)), resulting in a much more limited level of diversity.

Among the clones obtained from collections on estates, striking differences are also noted. Indeed, a very low level of diversity is observed within the samples of Caribbean populations such as **MAR** and **DOM**, whether due to an unscientific or inadequate method of collecting or to a high level of homogeneity within this material on the estates visited. By contrast, the high level of diversity observed within the **CL** population probably reflects the fact that this **Refractario** "population" is the result of the mixing of trees of three distinct genetic origins, as suggested by Pound (1943).

The level of heterozygosity was found to be low in the case of the two wild populations: **LCTEEN** and **GU**, while some **Trinitario** populations such as **UF**, **SC** and **CC** showed a high level of heterozygosity, which is not surprising due to the hybrid nature of the **Trinitario** group.

Diversity was partitioned "within" and "among" "populations", and the following results were obtained:

$H_{\text{within}} = 0.77$ and $H_{\text{between}} = 0.23$, when the Nei diversity index, based on allelic frequencies, was used;

$H_{\text{within}} = 0.61$ and $H_{\text{between}} = 0.39$, when the Shannon diversity index, based on genotypic frequencies, was used.

These data indicate that most of the diversity is due to variability within the "populations", which is not surprising for a

highly out-crossing perennial species (Hamrick *et al.*, 1992) like *Theobroma cacao* L., and which confirms the data obtained by other authors (Russel *et al.*, 1993; Ronning and Schnell, 1994).

These values show the need to maintain well-represented "populations" in cacao genebanks, although the necessary level of representation varies greatly among them.

Finally, the data obtained from a cluster analysis of the 28 cocoa "populations", based on Nei distances, using UPGMA method have been used to obtain the dendrogram shown in Figure 1. A rather clear separation between **Trinitario**, **Forastero** and **Refractario** "populations" was obtained, although some "populations", such as **IMC**, **ICS** and **CC** were misplaced. At the level of the **Trinitario** "populations", grouping was observed according to their geographical origin:

Trinidad (**ACT** and **TRD**);
Other Caribbean islands (**GS**, **MAR** and **DOM**) and;
Central and South America (**UF**, **SC** and **R**).

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dispersion are limited: "for Amazonia, and also for the South American continent, there are three uncontested main centres of plant dispersion which correspond to the Guyanan and Brazilian massifs and the Andean uplands...for Amazonian phytogeographic subdivisions of a lesser order, it makes sense to consider the floras of the different rivers and some groups of rivers which are floristically closely related" (Murça Pires, 1984).

Considering these arguments, **Map 1** can be proposed as a basis for work. The general genetic/taxonomic structuring of *T. cacao* would comprise 4 major natural groups (Trinitario, "artificial" hybrids, excluded):

- a) Criollo (Central America, Colombia, Venezuela),
- b) Amazons (currently called Upper-Amazon Forastero: Brazil, Peru, Ecuador, Colombia, Bolivia, Venezuela?),
- c) Guyanan (whole of the Guyanas' plateau: Venezuela, Guyana, Suriname, French Guiana, Brazil),
- d) Nacional (Ecuadorean Costa).

For the time being, we place cacao trees of the Lower-Amazon Forastero group (Comun, Pará, West African Amelonado, etc.) in the Guyanas (most probably Venezuelan).

Groups a) and d) would seem to have been separated from the others by the formation of the Andes, and groups b) and c) (currently making up the Forastero group) by events linked to Quaternary climatology.

In relation to these hypotheses, the following points ought to be investigated:

- the possible existence of a truly Lower-Amazon group (in the literal sense). This would require identification, characterization and integration of cacao trees reputed to be wild in the eastern zone of the basin (Obidos region, middle Tapajoz, lower Jarí, etc.?) in new analyses,
- an in-depth study of geographical origins (currently unknown) of the widely cultivated material known as Lower-Amazon (Comun, Pará, West African Amelonado, etc.),
- the symmetry and overall equivalent richness of the (Upper) Amazon and Guyanan groups; as the Guyanan group is under-represented in the collections and analyses, this will mean further surveys in the Guyanas plateau zones known to be populated with wild cacao trees, such as southern Guyana, certain regions of Suriname, maybe the Brazilian territory of Amapá, along with characterization of the material collected,
- the validity of the Nacional group, which is different from the Forastero and Criollo groups, and the possible indigenous origin (in Costa) of other Ecuadorean types (neither nacional, nor Trinitario) assumed to have been introduced by man from Amazonia (Pound, 1945),
- the precision of the Criollo-Amazon-Guyanan separation zones (Colombia and Venezuela).

In order to study these problems, we propose setting up a *Wild Cocoa Working Group* within *INGENIC*. If you are interested in this subject and you have theories or arguments to put forward or unpublished or little-known bibliographies, studies (or even clones) to propose, please contact the author.

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Assessing Genetic Diversity in the International Cocoa Genebank of Trinidad (ICG,T) using Isozyme Electrophoresis.

Olivier Sounigo, Romina Umaharan and Antoinette Sankar

The ICG,T contains roughly 88 cocoa «populations» (term designating each set of accessions obtained from the same collecting expedition or selected in the same research centre, in this article), whose representation varies largely in size, ranging from less than 5 to more than 200 genotypes. Isozyme Electrophoresis data obtained from five enzymatic systems (ACP, IDH, ADH, MDH and GPI), coded by six polymorphic loci, were used to estimate the genetic diversity existing within and among 28 cocoa "populations", represented in this study by 482 clones. These clones were characterized for at least 4 out of the 5 systems used, according to the genetic model proposed by Lanaud (1986).

Table 1 lists information about the "populations" studied, and Table 2 indicates the identity of the clones analysed. The data obtained for the different indices and variates used in the estimation of genetic diversity are shown in Table 3. This shows striking differences between "populations" for their level of genetic diversity. It has been verified that these differences in genetic diversity within the different "populations" were not due to differences in sample size (no correlation was found between the sample size of the "population" and any of the indices or variates used to estimate genetic diversity).

Genetic/Taxonomic Structuring of the *Theobroma cacao* L. species - Fresh Hypotheses

Philippe Lachenaud

Widespread use of biochemical and molecular markers over the last ten years or so to study the genetic structuring of the cacao tree has revealed the following:

- individualization of the Criollo group, warranting its classification a sub-species by Cuatrecasas (1964)
- the hybrid nature of the group called Trinitario,
- the individualization of wild cacao trees from French Guiana within the Forasteros,
- the heterogeneity of the group called "Lower-Amazon".

However, all the work undertaken in this field using "new" marking techniques suffers from two main drawbacks:

- the presumptions and errors concerning the basic groups (often transmitted by Cheesman, 1944) and the attribution of certain clones to those basic groups. For instance, it was thus that Nacional clones from Ecuador, wild trees from French Guiana, Venezuelan trees or even Upper-Amazonians were included in the Lower Amazon group.

- the unbalanced nature of the samples studied, which was serious in that the statistical analyses used were FAC, PCA or hierarchical classification.

There were consequently sometimes contradictions between the findings of different teams and enigmas (such as that of the Scavina clones) which confused breeders by convincing them that knowledge of cacao tree genetic structuring required further enlightenment.

We feel that in order to clarify this paramount aspect of genetic improvement, it would be important to take into account

other elements that can be used to put forward new hypotheses, which could then be confirmed or otherwise by one-off analyses using appropriate markers. In our opinion, the arguments to be considered are of the following type:

- Paleoclimatic

In the Quaternary period, especially the Pleistocene, the climates of the current cacao growing zone underwent considerable disruption: during the glacial periods, the whole of the zone has a much drier and cooler climate than now, which consequently pushed back the forest, and isolated or caused cacao populations to disappear (refuge theories). Major climatic disturbances have also occurred up to recent periods (around 3000 to 2000 years BP). Given its dispersal method, the cacao tree has not had time to recolonize the lost ground, which explains its current discontinuous distribution.

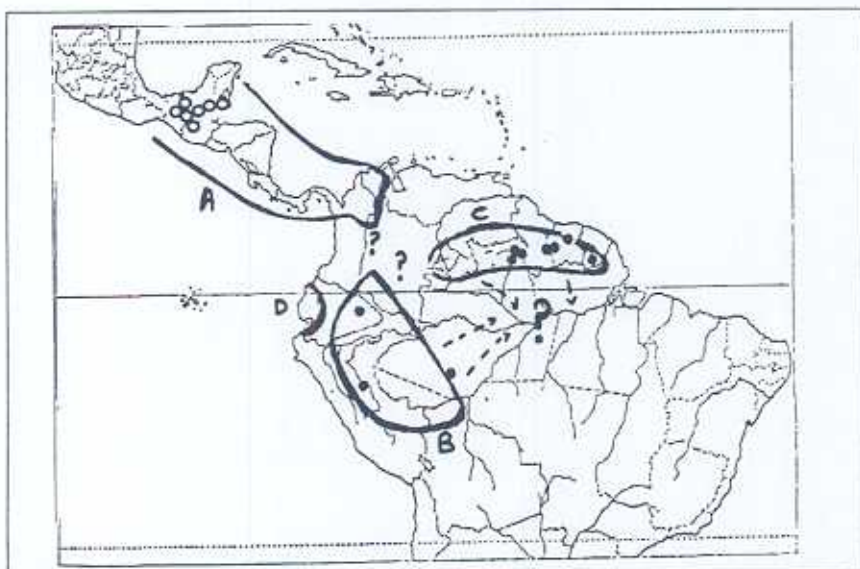
- Paleogeographic

Various theories show that a large central section of the current Amazon basin was in fact covered by water (fresh or salt) during the recent Quaternary (thereby making the existence of an original Lower Amazon group impossible) and that the Amazon and Orinoco basins were more interconnected than is actually the case (this is still the case with the Casiquiare).

- Geobotanical

What is known of the current distribution of wild cacao trees would make it possible to class this plant amongst type I peri-Amazonian species (North and West; de Granville, 1992). The cacao trees that currently occupy the lower part of the Amazon basin could thus originate from other regions upstream of the basin, "Upper Amazon" or southern slopes of the Guyanas plateau (and in this case transported there recently by rivers or by man, or from totally foreign zones e.g. the northern slopes of the Guyanas plateau, hence probably of anthropic origin).

Phytogeographical arguments need to be considered, all the more so for a plant such as cacao whose means of natural



Map 1: Approximate limits of the 4 major cacao groups mentioned in the text (A = Criollo, B = Amazon, C = Guyanan, D = Nacional). (Those marked with a '●' represent wild *T. sphaerocarpum*, those with a '○' *T. cacao*, recognized by Cuatrecasas).

Witches' Broom disease in Bahia

Dário Ahnert

Witches' Broom disease was first observed in the south-east of Bahia, Brazil, in 1990. It was then restricted to the counties of Camacan and Uruçuca. Recent surveys have shown that approximately 90 % of the 600,000 hectares of cacao in the region are already infected. The difficulty in controlling the disease and the decrease in production brought despair and discouragement to farmers, and they are now abandoning their plantations or removing the cacao and replacing it with pasture or other crops.

As a short-term solution, CEPLAC is recommending phytosanitary pruning to remove infected tissue, reduction in plant height, and regular pruning of plants to avoid contact between them and to facilitate broom removal and the application of fungicide to protect pods.

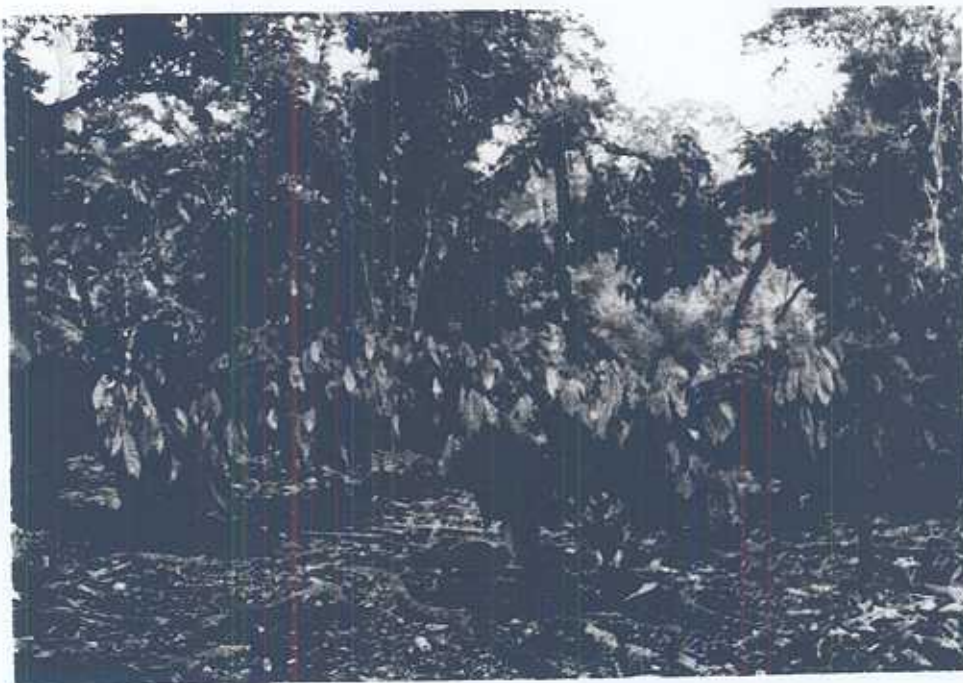
Unfortunately, many farmers cannot afford to implement these recommendations, and hence they are not controlling the disease. As a consequence, there is a high level of inocula in the region, which makes control difficult for the farmers who try to practise disease control. Another complication is the high susceptibility of the genetic material, *cacau comum*, which is grown on most of the farms. In the evaluation of the germplasm collection at CEPEC (Cocoa Research Centre), this group of accessions had the most vegetative brooms and an intermediate level of cushion brooms. The same results were observed in hybrid trials where progenies of *cacau comum* were evaluated.

As a long-term solution, CEPLAC is recommending resistant varieties. The first resistant hybrid variety named *Theobahia*, selected in regional trials, was released to farmers in 1994. This year, resistant clonal varieties, selected from regional trials, will be released to farmers for replacement of traditional cultivars. It

is known that substitution of old plantings by new varieties is not a common practice among farmers in Brazil. However, we expect to persuade farmers in Bahia to do so by showing them the potential of the new clones in demonstration plots, which are being established on cocoa farms in the region. In addition to the eight trials established by CEPLAC in the region, seven new ones will be established in a joint effort between CEPLAC and Fazenda Almirante (M&M Mars). These experiments have two-fold functions: to test new clones and to serve as an example to be followed by farmers to rehabilitate their plantations.

Evaluation for Witches' Broom resistance with the aim of obtaining new sources of resistance is underway. The evaluation of the germplasm collection is being done under medium inoculum pressure, which is maintained by the removal of brooms. This procedure is proving to be simple and effective, and has contributed to the identification of various promising genotypes and the characterization of resistance as an apparently quantitative trait. These resistant genotypes show wide genetic diversity with good possibilities of associating different genes related to resistance, which may contribute to widening of the level and stability of resistance. Information on germplasm evaluation was also used to define associations between origin and resistance, associations among different traits and to identify limitations of the sources of resistance such as small pod and seed size and low yields.

Assessment of the level and distribution of resistance as well as other traits in the germplasm collection is very important for the local breeding programme. Information is being used to consolidate the recurrent selection scheme in use at CEPEC, that involves one population formed by genotypes from the Upper Amazon, another involving Lower Amazon and Trinitarios (for the selection of progenitors from the next cycle), and a third one involving the incorporation of the others (for the selection of progenies and clones for commercial plantations).



Replanting with WB resistant material. (Photo: D. Ahnert)

In commercial plantations, about 1200 plants were identified as resistant and are being monitored for three years to confirm resistance. Selections within and among progenies in hybrid trials are underway to obtain superior progenies and clones. Those materials selected as resistant may be used either as clones for commercial plantations or in crosses to form new populations containing different sources of resistance. Therefore, much breeding effort is directed towards producing and releasing cultivars with the view to substituting old varieties and forming a new cocoa cycle in the region.

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Witches' Broom (Photo: T.N. Sreenivasan)

Was SCAVINA collected in the Ucayali River Basin?

Antonio Figueira

The outbreak of Witches' Broom disease caused by *Crinipellis pernicioso* in Southern Bahia, in 1989, has revived interest in the Scavina clones. The local Bahian variety "Comum" was shown to be highly susceptible (Pires *et al.*, 1996a), and the survival of the industry depends on substituting this traditional planting material with resistant genotypes. To date, Scavina 6 still remains a useful source of resistance in Southern Bahia. Based on the observation of natural infection due to Witches' Broom disease in the CEPEC/CEPLAC germplasm collection, which contains 565 accessions, fourteen genotypes from among the 18 with lowest disease

incidence were either Scavina 6 or 12 or their progenies (Pires *et al.*, 1996a). The low level of Witches' Broom infection, and the high yield observed in Amazonia and Southern Bahia led the CEPEC's breeders to release "Theobahia", a resistant hybrid variety derived from ICS 1 x Scavina 6 (Pires *et al.*, 1996b).

Historically, among the various plants introduced from Peru and Ecuador, which were collected by Pound, only Scavina 6 and 12 were observed as remarkably resistant (Bartley, 1986). Due to the poor agronomic characteristics, these genotypes were not directly used as clones, but were crossed to develop single-cross hybrids and clones. With the realization that crosses involving parents of different geographical origin led to hybrid vigour, Scavina 6 and 12 were frequently used as parents in bi-parental hybrid crosses in most of the cocoa breeding programmes, mainly in the Americas. As clones, the Trinidad Selected Hybrids (TSH), selected from crosses containing Scavina 6 as one parent, have been used commercially in Trinidad, and appeared to have had a major role in the reduction of the incidence of Witches' Broom disease there (Laker *et al.*, 1988). Some of these TSH clones are earmarked for distribution by CEPLAC in Southern Bahia in the near future. Scavina 6 and 12 were still considered resistant in Trinidad in 1988 (Laker *et al.*, 1988), however infection symptoms have been observed since 1976 (Bartley, 1986). In Pará, Northern Brazil, Scavina 6 and 12 were initially found resistant (Evans and Bastos, 1980), but the vertical resistance broke down within a few years, similar to what occurred in Ecuador and Bolivia (Bartley, 1986). Therefore, the endurance of Scavina 6 resistance is unpredictable, considering that variation in resistance reaction is due to pathogen diversity.

Despite its importance on the history and progress of cocoa breeding, little is known about the real origin of Scavina. From the original report of the expedition to the headwaters of the Amazon river in 1937-38, Pound (1938) described five basic cacao populations sampled, based on pod morphology: 1. "Nanay" (long oval pod); 2. "lagarta" or "Parinari" (long warty pod with pronounced bottle-neck and conspicuous apex); 3. "lagarta calabacillo" from an island across from Iquitos (large oval and smooth pod); 4. "lagarto colorado" from the Napo river (light green warty "lagarta" with splashes of anthocyanin on the ridges); and 5. Ucayali river (green, moderately warty pods). These types were kept separate but not as individual trees, and 250 pods were sent to quarantine in Barbados (Pound, 1938). Despite the description of the material, there have been controversies about the exact location of Scavina collection. Hardy (1961) suggested that the Scavinas were collected as a single pod from an Ecuadorian plantation (thus as a "refractario"). However, Posnette (1982) claimed that "Dr. Pound undoubtedly wrote that the Scavina were indeed collected somewhere in the Upper Amazon." Traditionally, many authors have referred to the Scavina genotypes as having originated in Ecuador. It is noteworthy that the borders between Ecuador and Peru are different today from those existing during Pound's expeditions. Bartley (1986) considered Scavina 6 and 12 to belong to a single progeny from a collection made between Contamana and Pucallpa on the Ucayali river. In fact, Pound collected and sent to Trinidad pods collected near Contamana on the Ucayali river (Pound, 1938). Pound (1943) returned to Peru, and collected budwood from one disease-free tree, originally from the Ucayali river, and named it P 31 or Pound 31.

The imprecise knowledge of the collection site of the Scavinas has hampered the re-sampling of the same area. Recently, cacao collections in the Ucayali and Huallaga river basins were conducted under the leadership of Mr. F.J. Coral, sponsored by a

UNIDO project. Mr. F.J. Coral made extensive collections throughout the Ucayali and Huallaga river basins in 1987-89 in search of Witches' Broom and Moniliasis resistant plants. These collections are being maintained at the Facultad de Agronomía of the Universidad Nacional Agraria de La Selva, in Tingo María, Peru (Rios, 1995), and at the Estacion Experimental de Sahuayacu, Echarati, Peru. Some of these genotypes were introduced into Brazil, and are located at the Instituto Agronomico de Campinas, (Campinas, SP). A few genotypes were later brought to the Almirante Centro de Estudos de Cacau, (Itajuípe, BA), Unacau farm (Una, BA) and CEPEC/CEPLAC (Ilhéus, BA).

A small sample of these genotypes were used to examine the relationship among the Ucayali and Huallaga genotypes and Scavina 6, in order to determine the approximate area of origin of Scavina so as to enable further collections in the same area (Figueira *et al.*, 1996). Five Ucayali clones, 11 Huallaga clones, and Scavina 6, along with two non-related genotypes (Pa 150 and Ma 15) were analyzed by the RAPD technique, using 18 arbitrary primers, and 96 fragments were scored. Some primers amplified fragments typical of either Ucayali or Huallaga populations. The overall average genetic similarity among the 19 genotypes was 70.4%. The genetic similarity within the Ucayali genotypes was smaller ($74.9 \pm 2.5\%$) than among the Huallaga ones ($78.1 \pm 0.8\%$). The genetic similarity among all the Ucayali clones and Scavina 6 was 77.9%, while that between the Huallaga clones and Scavina 6 was 60.2%. The similarity between Scavina 6 and Pa 150 was 66.7%, while the similarity between Scavina 6 and Ma 15 was only 57.1%. The genotypes Ucayali 6 and 12 were the most similar to Scavina 6 (84 and 82.6%, respectively). Based on the genetic similarity data, genotypes were grouped using cluster analysis. Two clearly distinct groups could be identified: one group contained all the Huallaga genotypes plus Pa 150 and Ma 15. The second group contained all the Ucayali clones and the Scavina 6. Both groups were very heterogeneous, despite the fact that the genotypes grouped according to the river basin of origin (Ucayali and Huallaga). The genotype Scavina 6, as well as other genotypes from Acre, Brazil (CSUL and RB), have been shown to display low genetic similarity to most of the other genotypes, when analyzed by molecular markers.

The genotype Ucayali 6 was claimed by Mr. F.J. Coral to have been collected near the putative original site of collection of the Scavina genotypes. According to Mr. F.J. Coral, Scavina might have been collected near Contamana, at the "Fundo Monte Blanco" farm (S7°20'; W75° 01', 110 m a.s.l.), left margin of the Ucayali river, owned by Mr. Armando Facchin, successor of Mr. Eduardo Scavino (F.J. Coral, pers. comm.). The Ucayali genotypes were closer to the Scavina 6, but it was a heterogeneous group, and could be considered a potential alternative source of tolerance. In fact, preliminary results indicated that genotypes Ucayali 10 and 54 presented Witches' Broom tolerance, when evaluated under field and greenhouse conditions (budded seedlings) with artificial inoculation (Rios, 1995). The genotypes from Ucayali demonstrated more tolerance to Witches' Broom than the Huallaga genotypes (Rios, 1995). Further evaluation for Witches' Broom tolerance are currently underway in Peru, and in Brazil at Almirante Centro de Estudos de Cacau, and at CEPEC/CEPLAC.

In conclusion, the analysis of genetic similarity based on RAPD markers suggested that, indeed, Scavina 6 might have been collected in the Ucayali river basin because of its closer resemblance with genotypes from the same area. The genotypes from the Ucayali formed a heterogeneous group, distinct from the Huallaga genotypes, and the two control genotypes (Pa 150 and

Ma 15). Further studies are required to confirm the potential use of these genotypes as a source of Witches' Broom and Moniliasis resistance.

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Brief Report on Collection of Wild Cacao from the Euleupousing and Yaloupi Rivers in French Guiana

Philippe Lachenaud (CIRAD-CP), Vishnarayan Moolleedhar (CRU), Christophe Couturier (CIRAD-CP)

This is an account of the third collecting expedition in French Guiana organized by CIRAD. It was part of a cooperative project between CIRAD (French Guiana) and the Cocoa Research Unit (CRU) of the University of the West Indies, Trinidad to study wild French Guianan cocoa. It was the first international collection expedition involving CIRAD and CRU. Funding was provided by *Fonds Interministériel Caraïbe-FIC, France*.

The survey covered the banks of the Euleupousing and Yaloupi rivers, tributaries of the upper Oyapok (extreme south eastern French Guiana) and the Oyapok itself, between the two tributaries. It was carried out from April 13th to 26th, 1995; i.e. during the fruiting period, which coincided with the rainy season.

We left Camopi, halfway down the Oyapok, in the morning of April 13th. The low water level, which was unusual for the time of the year, slowed down our progress and our arrival at the confluence with the Euleupousing, which was scheduled for the evening of the 13th, was delayed until the afternoon of the 14th.

Euleupousing River (14/4 to 20/4/95)

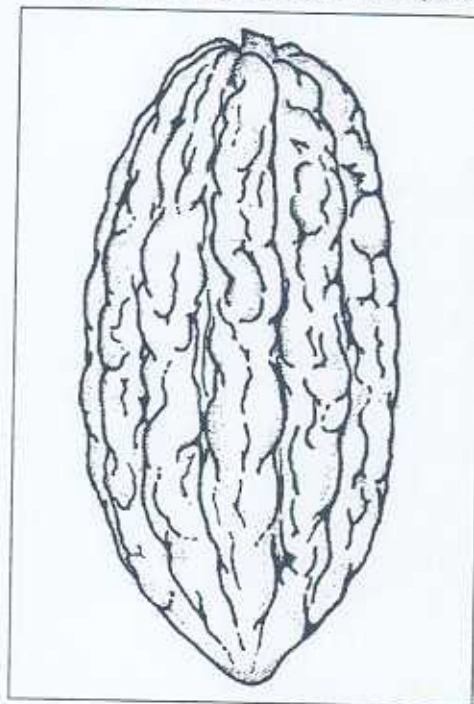
This rather minor river (by Guianan standards!) has only one serious obstacle and that is the Boko Falls with a drop of five metres, some 10 km from the confluence with the Oyapok. However, the river is so narrow after this point that it is constantly blocked by fallen trees. Between April 14th and 17th, we travelled the main river upstream and also certain tributaries, recording the position of cacao trees, from which we collected on our way back downstream on the 18th and 19th of April. Once the river was no longer navigable, we continued for some 5 km on foot towards the Mont St. Marcel, but failed to find any more cacao trees.

The zone with cacao trees begins a few km upstream of Boko Falls, ending slightly upstream of Cambrouze falls, i.e. around 10 km as the crow flies, but much further along the river, with its numerous meanders. We collected from 26 mother trees, 22 in the form of pods (21 are represented in French Guiana and 15 in Barbados). Four trees were collected in budstick form and budded in French Guiana (4) and Barbados (1). The material collected is coded as ELP (1 to 41).

Of these 26 trees, eight were growing isolated and the others were in groups of three to 50 trees. Their approximate average height was 11 m, ranging from 4 to 25 m, with an average canopy width of 8 m. Nine trees had a single stem, five had a few stems and 13 had clusters of stems. The mean distance from the river to the trees was around 20 m, varying from 0 to 100 m. The mother trees had mostly pods of Amelonado type shape (59%) or "Guianan" (32%) type (intermediate between Amelonado and Anjoleta, see drawing). The pod surface ranged from smooth to slightly rough. No sign of Witches' Broom and very few rotten pods were seen. A few pods contained pale coloured beans.

The weather was relatively good for the time of year during our trip, which was nevertheless extremely action-packed. We are not likely to forget Vishnarayan's fall into the rapids at Boko Falls,

when he swallowed a good mouthful of the river (14/4) and a somewhat unexpected 500 m swim at nightfall (18/4) for Philippe and Christophe, in the strong currents of the Euleupousing to reach the camp with their pockets stuffed with pods!



Guianese pod shape

Oyapok River (20/4)

Between the confluences of the Euleupousing and the Yaloupi, material was collected from a few trees seen on the banks. Only one had pods (Amelonado type), and two were collected as budwood. This material is coded as OYA (1 to 4) and is to be compared with the PINA material collected upstream on the same river in 1990. We noted a suspect case of Witches' Broom on a cacao tree on the Brazilian bank (not collected).

Yaloupi River (21 to 26/4)

This tributary of the Oyapok, which is much larger than the Euleupousing, is, like the latter, the subject of a taboo on the part of the Indians because of the supposed presence of other dangerous Indians. This taboo and the difficulty of travelling upstream make the river and its banks a veritable sanctuary. In fact, along the first 20 km from the confluence with the Oyapok, three difficult falls (particularly Walmikoualé, with a six meter drop) dissuade, if not prevent travel. We travelled up river for three days until the evening of 23/4, when various incidents and the fact that we were due to fly home prevented us from going any further.

If there are large populations of cacao trees along the river, they must be near to the source, as we saw very few downstream. Only five mother trees were collected, four of which had pods, at three sites upstream of Polissoirs falls. The material collected is known as YAL (1 to 8). The pods were smooth and of Amelonado (50%) or Calabacillo (50%) shape. We did not see any signs of Witches' Broom.

Conclusion

In short, the scientific result of the collection mission is very positive since 34 mother trees representing three very distinct populations were collected. Unique characters were noted such as the Calabacillo shape and a few pods with pale beans. We confirmed the absence of Witches' Broom disease. The surviving material has been planted in the CIRAD collection in French Guiana, near Kourou, and is due to be planted in Trinidad after quarantine in Barbados hence ensuring its preservation.

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Crossing Pound's Trail in Tingo Maria, Peru

Hille Toxopeus

In the course of an advisory mission for the Netherlands Management Consultant Project (NMCP) to the Genetics and Plant Breeding Department of Universidad Nacional Agraria de la Selva (UNAS) in Tingo Maria (TM) in March/April this year, I was unexpectedly confronted with a remarkable cacao population derived from the Pound (P) clones. The plantations visited were 40-50 years old and several had recently been cut down through lack of profit. The intact plantations consisted of widely spaced, giant trees with a closed canopy 4-6 m high. The pod type of this population can best be described in Pound's terminology:

The central type is a medium to large, oval, half blanco, slightly lagarto pod, possessing neither conspicuous point nor bottleneck; varying from blanco to green in colour and from smooth and pointed to lagarto.

Farmer Reyes from TM remembers having assisted his father in the early '50's with the planting of his presently 45 odd year-old cacao plantation with Pound's seedlings (as he said) from INIA's TM Experimental Station where, apparently, the Pound clones were podding to such an extent that farmers were planting it. The resulting variety is referred to as the Pound's variety.

Pound stated in his 1943 report that he left the P clones established as 4 budlings each, planted in INIA's Iquitos Experimental Station in 1942. It looks as if INIA multiplied the clones (all, or in part? - This is yet to be confirmed) to its Experimental Station at Tingo Maria, presumably also as budlings on presumably red-podded rootstocks (to be confirmed).

The above pod description is almost exactly that of the Nanay population in Pound's 1938 report, paragraph 114. Most of the P clones were derived from the Nanay population (Pound 1943, p. 9-10). The other group of selected trees of the "Napo" population could not be distinguished from Nanay by pod type, but by tree habit, several of which had a "weeping" jorquette with fan branches growing out horizontally instead of upwards (Pound, 1943, p.7). Pound emphasizes this trait because he had observed it in resistant trees in Ecuador. Strikingly, in old and cut down Pound

variety plantings around Tulumayo (TM) several tree stumps were observed growing chupons with a "weeping" jorquette.

These matching descriptions make it more than likely that the early plantings from the TM clonal garden were derived from purely Pound clones, i.e. the garden contained only Pound clones. In time, other so-called "international" clones were added, broadening the mix (and adding susceptibility to Witches' Broom disease).

The plantations of the Pound variety have resisted WB attack over the years to a remarkable extent and I consider it field resistant to WB attack of shoots (canopy), but susceptible trees are segregating. However, it is quite clear that fruits are susceptible and this, combined with *Monilia* podrot that came in a year or two ago, has eventually made these plantations practically redundant. The variety has otherwise shown good adaptation to local conditions and satisfactory quality. It has proven to be dependable and therefore worthwhile conserving since it is now under threat of erosion. It is worth further genetic improvement.

The Genetics and Plant Breeding Department of the Faculty of Agronomy, UNAS carries national responsibility for cacao germplasm in Peru. It is planned to conserve the Pound variety as a population, in terms I described earlier in this newsletter, and in the *Cocoa Research Newsletter Issue 3*.



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.



The INGENIC Committee wishes to bid farewell to Professor John Spence as Head of the Cocoa Research Unit, the University of the West Indies, Trinidad & Tobago, and to welcome the new Head, Dr. David Butler.

Obituary

Simeon Abiodun Akinwale

The late Dr. S.A. Akinwale was born on December 31st, 1957 at Edunabon, Ife. He had his primary education at St. Peter's Primary School, Edunabon, and attended Origbo Grammar School, Ipatumodu, where he rose to the position of School Captain (head prefect) during his final year. He later proceeded to the University of Ife (now Obafemi Awolowo University) where he obtained his B.Sc. (Hons) (Botany) in 1983, M.Sc. (Genetics) in 1987 and Ph.D. (*Biosystematics*) in 1990.

He was an employee of NEPA, Lagos after his secondary education. He joined the service of the Cocoa Research Institute on May, 22nd 1987 as a Research Officer Grade I. Through hard work, he rose to the posts of Senior Research Officer in 1991 and Principal Research Officer in 1992, the post he held until his death on May, 31st, 1996, three days after his involvement in a motor vehicular accident.

He was quite active in many spheres of endeavour. At the time of his death, he was the *inter alia* General Secretary of the Genetics Society of Nigeria, Chairman of Full Gospel Businessmen's Fellowship International (Oluyole Local Government Chapter) and Assistant Pastor of his church - The Redeemed Christian Church of God, CRIN Parish.

He is survived by his wife and two children. May his gentle soul rest in perfect peace.



FORTHCOMING EVENTS



- (1) 1 Congreso Venezolano Del Cacao y su Industria
18 - 21 November 1997

Hotel Maracay,
Aragua State,
Venezuela.

For information contact:

Congress Organisers at e-mail address:
ccacao@chroni.fundacite.arg.gov.ve
Tel. 043-320966/324791/323244

OR In Caracas: Tel: (02) 7312942/7312660

- (2) 2nd International Seminar on the Diseases
and Pests of Cocoa
19 - 24 January 1998

Yamoussoukro, Côte d'Ivoire

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Explanation of acronyms

ACRI	- American Cocoa Research Institute
BCCCA	- Biscuit, Cake, Chocolate and Confectionery Alliance (United Kingdom)
CAOBISCO	- The European Employer's Federation for the Chocolate and Biscuit Industry
CEPLAC	- Centro de Pesquisas do Cacau
CPA	- Cocoa Producers' Alliance
CIRAD	- Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CRIG	- Cocoa Research Institute, Ghana
CRU	- Cocoa Research Unit
CTA	- Technical Centre for Agricultural and Rural Cooperation
FAO	- Food and Agriculture Organisation of the United Nations
IDEFOR-DCC	- Institut des Forêts Recherches Agronomiques en Zone Forestière - Département de CAFE-CACAO et Autres Plantes Stimulantes
IOCCC	- International Office of Cocoa, Chocolate and Sugar Confectionery
IRAD	- Institut de Recherche Agricole pour le Développement

STAMP