THE UNIVERSITY OF THE WEST INDIES

COCOA RESEARCH UNIT

REPORT FOR 1995

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ST. AUGUSTINE, TRINIDAD & TOBAGO

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BIOCHEMICAL CHARACTERIZATION

Overview of the Activities of the Biochemistry Laboratory

O.Sounigo, Y. Christopher and A. Sankar

This year, characterization and genetic diversity assessment have been continued, using RAPD and isozyme electrophoresis techniques. The value of RAPD technique for finger printing has been assessed by evaluating its reproducibility. In addition, a project has been initiated in June 1995, in order to:-

- map cacao genome and search for molecular markers linked to resistance to *Phytophthora*
- accumulate alleles confering resistance to the same disease in progenies originating from several cycles of crosses between resistant genitors.

This project, financed by CAOBISCO Involves the collaboration of CRU with several other research institutes such as CIRAD (France), IRA (Cameroun) and IDEFOR (Ivory Coast).

Evaluation of the reproducibility of the RAPD technique.

The reproducibility of this technique has been assessed, comparing experiments conducted in the same laboratory or in two different laboratories (biochemistry laboratory of the C.R.U and AGETROP laboratory, at CIRAD (France). The experiments repeated in the same laboratory showed that the model of thermal cycler is not important, once the same amplification programs are performed. These experiments were also conducted to evaluate the impact of the age of DNA extracts. In our case, in which relatively impure and degraded DNA is obtained, the extracts cannot be kept over seven months without the risk of modification of the banding patterns.

More details are given in the following paper "The use of RAPD for characterization and genetic diversity assessment of cocoa.", by Y. Christopher and O. Sounigo.

Reproducible results could be obtained between the two laboratories, only after a severe screening of the markers to be considered. More details are given in another paper " Evaluation of the reproducibility of RAPD." by O. Sounigo and Y. Christopher.

Fingerprinting

Using six enzymatic systems, 90 clones have been characterized allowing the obtention of 61 different fingerprints. Fifty-seven clones were uniquely fingerprinted.

Using RAPD (18 markers used), 47 clones have been characterized allowing the obtention of 35 different fingerprints. Twenty-seven clones were uniquely fingerprinted. More details are given in the two following papers:

"The use of RAPD for characterization and genetic diversity assessment of cocoa.", Y. Christopher and O. Sounigo, and "The use of isozyme electrophoresis for characterization and genetic diversity assessment of cocoa.", A. Sankar and O. Sounigo.

Genetic diversity assessment.

One hundred eighty-nine clones, representing 14 populations and 89 clones, representing 11 populations, were analyzed, respectively by the use of isozyme electrophoresis (5 enzymatic systems) and RAPDs (12 markers obtained from 5 primers). Eight of the populations were used in both types of analysis, as shown in the Table 1.

Concerning the clustering of the different populations (figures 1 and 2), some similarities are found, such as the tight clustering of the different Trinitario populations (UF, ICS, GS and RIM) and the clustering of some populations collected by POUND in Peru (NA, P and PA). In addition, SCA population appears to be relatively differentiated from the other populations originated from Pound's collection in Peru, in both studies. On the other hand, some differences are found, at the level of the clustering of the B, SPEC and IMC populations. Indeed, SPEC and IMC populations are found to cluster with the Trinitario populations, according to the results obtained using isozyme electrophoresis, while these populations cluster with the B population, clearly separated from the Trinitario populations, according to the results obtained, using RAPD.

Concerning the diversity within populations, some similarities are found, such as the relatively high level of genetic diversity within the B and the SCA populations, and such as the low level of diversity found in IMC and P populations. On the other hand, differences are found at the level of the PA population, showing a high level of diversity after the RAPD study and a low level of diversity after the isozyme study.

However, the results shown here are far from definitive and have to be completed by increasing the number of clones per population, especially in the case of the RAPD study and by increasing the number of markers, which will be very difficult in the case of isozyme electrophoresis, since attemps have already been made in order to perfect new enzymatic systems, without noticeable results. Another limitation to the comparison between the two studies is that the populations analyzed by both techniques were not represented by exactly the same clones, because of problems of flush leaves availibility, required for isozyme electrophoresis.

More details are given in the two following papers:
"The use of RAPD for characterization and genetic diversity assessment of cocoa.", Y. Christopher and O. Sounigo, and "The use of isozyme electrophoresis for characterization and genetic diversity assessment of cocoa.", A. Sankar and O. Sounigo.

Genome mapping and search for markers linked to resistance to *Phytophthora*.

A program has been inititiated in June 1995, in order to map cocoa genome and to search for molecular markers linked to resistance to *Phytophthora*. These markers could be then used in Marker Assisted Selection programs.

Crosses have been chosen, in such a way that they involve a female genitor with a high level of resistance to *Phytophthora* (determined by leaf test) and a high level of heterozygosity (determined by RFLP and isozymes), on one hand, and a male genitor combining a high level of susceptibility to Phytophthora (determined by leaf-test) and a low degree of heterozygosity (determined by RFLP, isozymes and RAPD).

The following female genitors have been chosen:
ICS84, IMC57 and TSH1077. Despite their lower level of heterozygoty, GU175 and SCA6 have also been used as female genitors.

CATONGO has been chosen as a male genitor.

All the progenies are now in nursery but only two of these progenies will be used for genome mapping and research of markers linked to resistance, after having been chosen for their suitability for this kind of study, i.e their ability to allow a segregation at both RAPD markers and resistance to *Phytophthora* levels.

Pre-breeding

A program has been initiated in order to accumulate alleles confering resistance to *Phytophthora* in progenitors originated from several cycles of crosses among resistant genitors.

The crosses have been performed between two genitors which have been found to be resistant after studies using a leaf test. The following crosses have been performed:

IMC 57 x TSH 1077, ICS 84 x TSH 1077, SCA 6 x GU 175, SCA 6 x ICS 45, SCA 6 x ICS 84, B 721 x IMC 103, NA 45 x IMC 57, NA 45 x GU 175 and NA 45 x B 721.

All the progenies from these crosses are now in nursery and all the plants will be studied for their level of resistance to *Phytophthora* using leaf tests. The most resistant plants of each family will then be planted, in order to study their resistance on pods (after both artificial and natural infection). The most resistant trees will then be used as genitors in an additional breeding cycle, in order to accumulate the alleles confering resistance to *Phytophthora* in the resulting progenies.

Table 1: Populations studied by RAPDs and Isozyme Electrophoresis.

population	sample size for RAPD study	sample size for Isozyme study	Number of clones studied by both techniques
B	8	12	5
ICS	14	23	8
IMC	10	25	6
NA	8	30	3
PA	7	17	2
P	6	11	3
SCA	11	8	7
SPEC	7	10	3

Fig. 1. Results from Isozyme Electrophoresis. Dendrogram obtained after a clustering analysis based on Nei and Li coefficients calculated from the frequencies of the different alleles of each locus in each population.

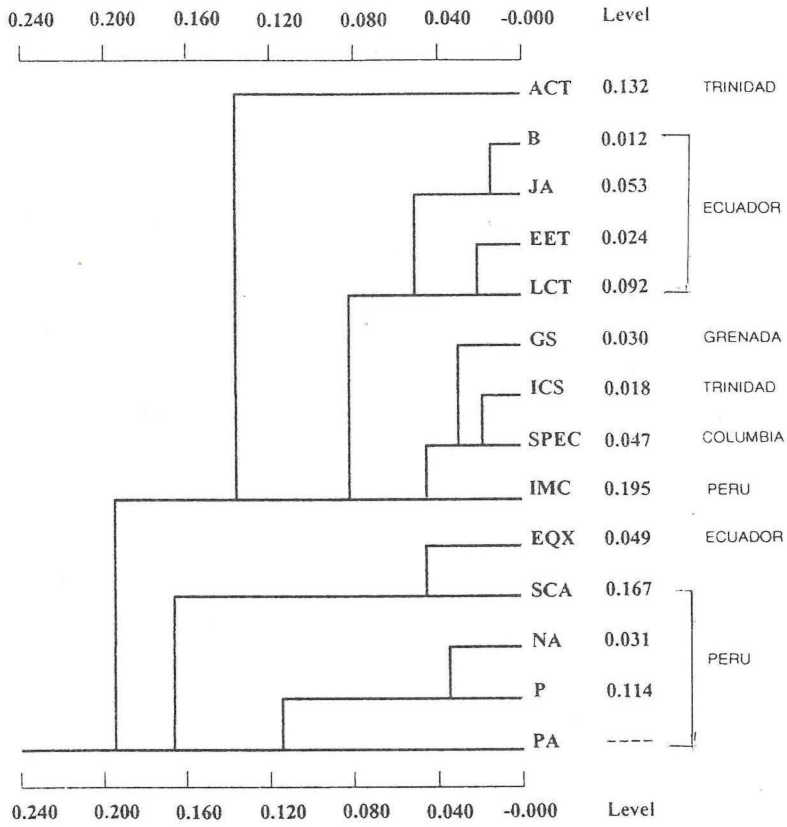

Fig. 2. Results from RAPD. Dendrogram obtained after a clustering analysis based on Nei and Li coefficients calculated from the frequencies of the different markers in each population.