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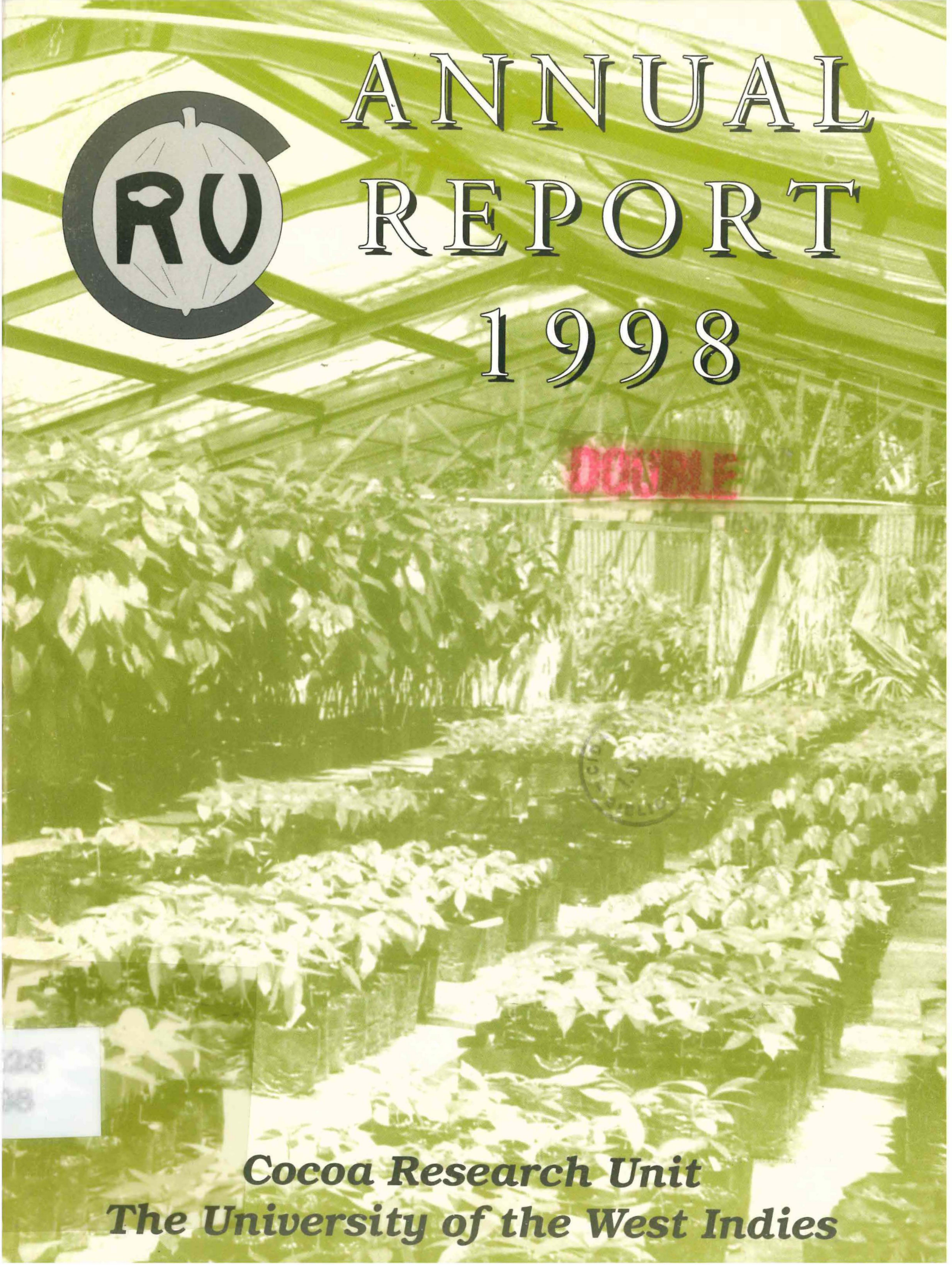

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Cover photograph. Cacao seedlings in the germplasm enhancement programme.

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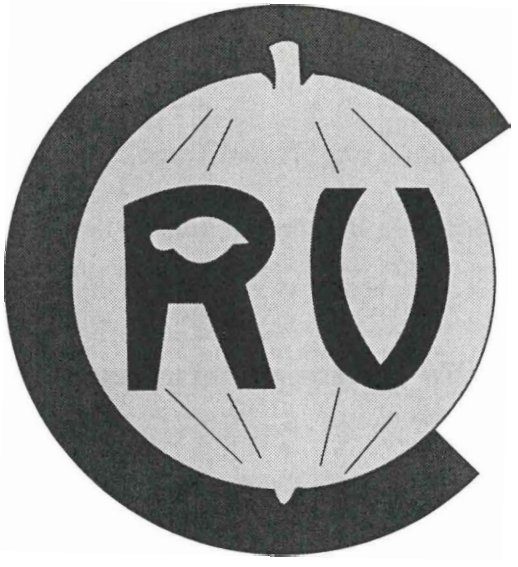

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Assessing Cacao Genetic Diversity Using IE and RAPD Techniques

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Introduction

Accessions in the ICG,T are being analysed with biochemical (IE) and molecular (RAPD) techniques, to assess the level of genetic diversity in the genebank and within each represented population. Genetic relationships between different populations are analysed, to describe the structure of the genetic diversity in *T.cacao*. The results are being used to search for potential heterotic groups and may assist in the choice of a subsample from the ICG,T for a core collection.

To date, 508 clones, representing 30 populations have been analysed with all the five enzymatic systems used routinely at CRU and 292 clones, representing 13 populations, have been analysed using 41 RAPD markers obtained from 17 decameric primers.

Results

The RAPD data were used to evaluate the level of diversity in each of the populations studied, using the Shannon Index (Table 1), which is based on the number of polymorphic markers and on their relative frequency. No correlation was found between Shannon Indices and sample sizes.

Table 1. Level of genetic diversity found in 13 cacao populations from the ICG,T shown by Shannon Indices, calculated from RAPD data

Population	Origin	Sample size	Shannon Index
B	Ecuador (Hacienda Balao)	15	0.27
CL	Ecuador (Hacienda Clementina)	8	0.34
ELP	French Guiana (Elepoussing River)	9	0.22
GU	French Guiana (Camopi River)	19	0.15
ICS	Trinidad	18	0.31
IMC	Peru (Iquito)	21	0.33
JA	Ecuador (Hacienda Javilla)	19	0.28
LCTEEN	Ecuador	17	0.39
MO	Peru (Morona River)	13	0.33
NA	Peru (Nanay River)	22	0.28
P	Peru	17	0.36
PA	Peru	20	0.32
SCA	Peru (Ucayali River)	13	0.36

The least diversity was found in GU and ELP, two populations from French Guiana, but the ELP population appeared a little more diverse, this trend being confirmed by data from IE in French Guiana and at CRU. The greatest diversity was found in:

- LCTEEN, to be expected with cacao trees collected from a very large area

- CL, due to the different genetic origins (Nacional, Trinitario, and Forastero) of this cultivated material
- ICS, also expected due to the hybrid origin of this population
- IMC and PA is rather surprising, especially IMC which is thought to originate from only two mother trees (Lockwood and End, 1993), and shows a low level of morphological diversity
- MO is very surprising, since these clones are thought to originate from a single mother tree. One possible explanation for this high level of diversity could be a mistake; some MOQ clones from Ecuador having been accidentally named MO (Bartley, quoted by Wadsworth *et al.*, 1997).
- P is not surprising, since these clones are the mother trees of the different populations collected by Pound in Peru.
- SCA is very surprising, since this population is thought to originate from a single mother tree (Lockwood and End, 1993).

Figure 1 depicts genetic relationships between the different populations (cluster analysis performed on Rogers-Wright distances calculated on RAPD data). As expected, the two populations from French Guiana cluster together and are genetically distinct from all the other populations. These two populations contain a specific RAPD marker, found in no other population. The IMC and NA populations also cluster together, which is not surprising, since they originate from mother trees growing in the same area. The two Refractario populations B and JA cluster together, indicating that very similar cacao was planted in the two haciendas from whence these populations were collected. The genetic dissimilarity between the P and NA populations is rather surprising, since NA clones are thought to originate from 14 mother trees in the P population (Lockwood and End, 1993). This result contrasts with that observed when IE is used (Figure 2) where there is a close similarity between these two populations.

Figure 3 illustrates the results from a Principal Component Analysis. It shows that most of the SCA clones analysed are similar to P 31A, confirming the hypothesis that P 31 could be the mother tree of these clones (Lockwood and End, 1993). Most of the MO clones appear to be different from the other populations collected in Peru. One possible hypothesis to explain this result would be the statement made by Bartley (Wadsworth *et al.*, 1997) that some of the MO clones are in fact MOQ clones, MOQ being a population from Ecuador.

Figure 1. Dendrogram depicting the genetic relationships between 13 cocoa populations obtained from a cluster analysis (Unweighted Pair-Group Method using Arithmetic averages) performed on Rogers-Wright distances calculated on RAPD data

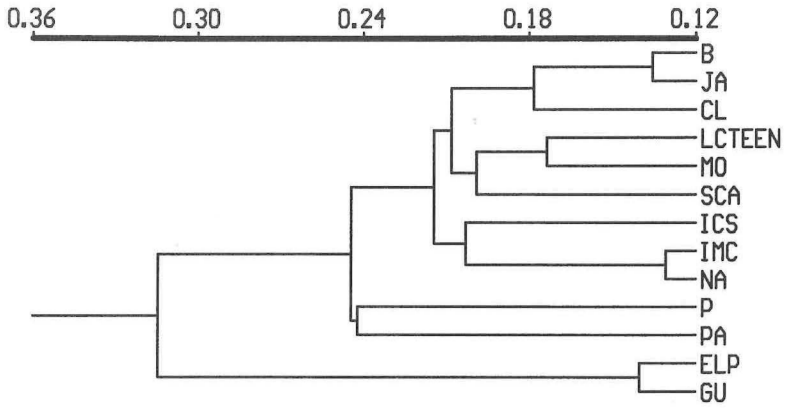


Figure 2. Dendrogram depicting the genetic relationships between 30 cocoa populations represented in ICG,T, obtained from a cluster analysis (Unweighted Pair-Group Method using Arithmetic averages) performed on Nei distances calculated on isozyme electrophoresis data

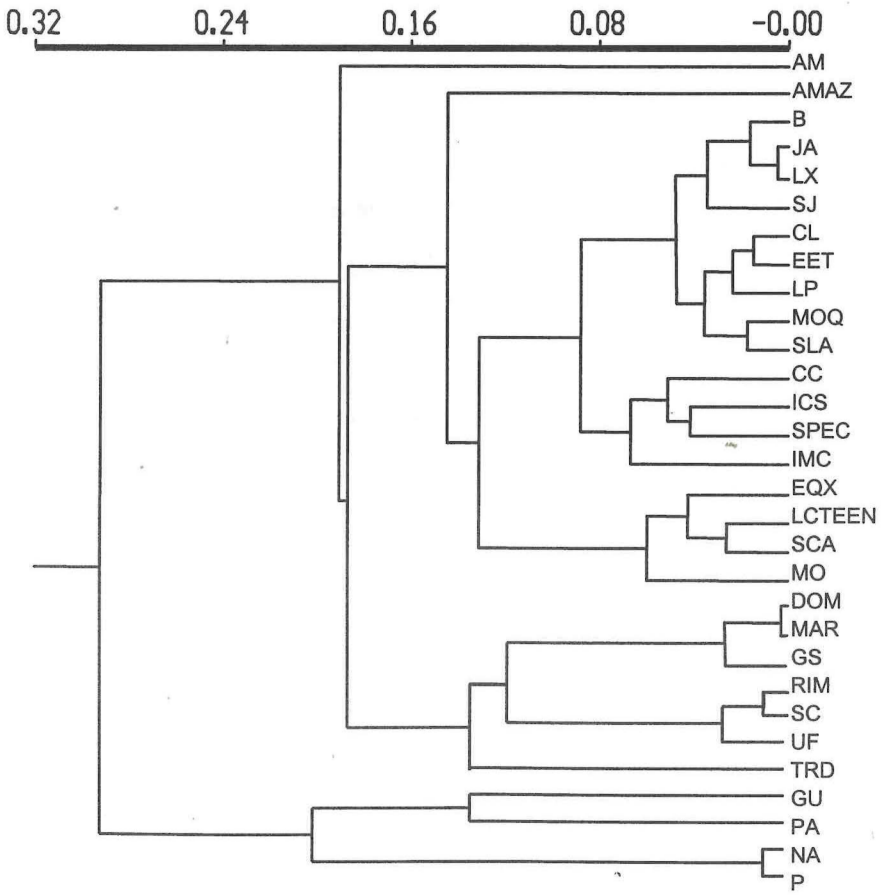
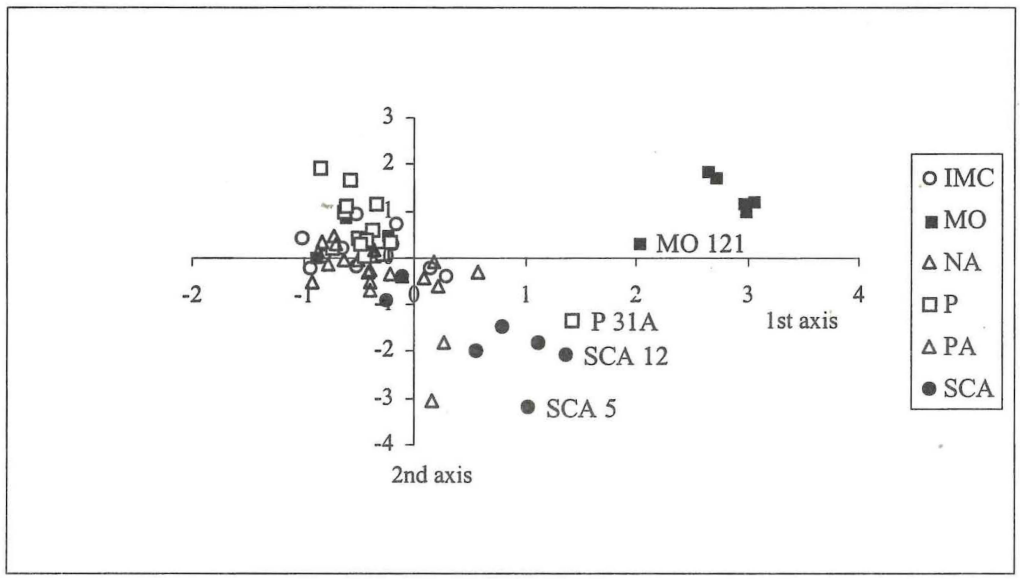


Figure 3. Plane defined by the two first axes of a Principal Component Analysis performed on RAPD data obtained from 13 cocoa populations represented in the ICG,T. The first axis explains 15% and the second 10% of the total diversity



Future prospects

The genetic diversity studies will be continued, using both RAPD and IE, to study additional populations. Very soon, RAPD data will be available for AMAZ, EQX , UF, GS, and EET populations. The data will be used as a guide to establish a priority list of clones for the CFC Cocoa Project and in attempts to confirm various hypotheses on the origin of some populations.

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