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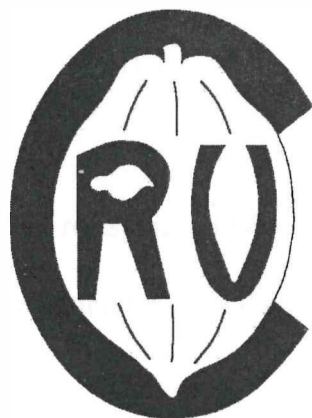
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Cover photograph. Progeny from the cross of ICS 1 x SCA 6 planted in the 1950s.

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Genetic diversity assessment using RAPD

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Introduction

RAPD characterisation of cocoa accessions was started in 1994 and, so far, 615 accessions have been completed. This year, accessions were chosen in order to complete information on some Trinitario populations (DOM, RIM, SC, TRD) and to characterise the accessions pre-selected for the CFC/ICCO/IPGRI project collection. The data are used to examine the genetic relationships between accessions within some of the populations.

Results

Newly analysed Trinitario populations

Table 1 shows large differences in the level of genetic diversity observed within the newly analysed populations. The TRD population (accessions collected in several cocoa estates in Trinidad) shows a very large level of genetic diversity, contrasting with the RIM and SC populations (accessions selected in Mexico and Colombia, respectively). The DOM population (accessions collected from estates in Dominica) shows a rather low level of genetic diversity compared to the TRD population, which was collected in a similar manner.

Table 1. Shannon indices (H_i) for four Trinitario populations, newly analysed using RAPDs.

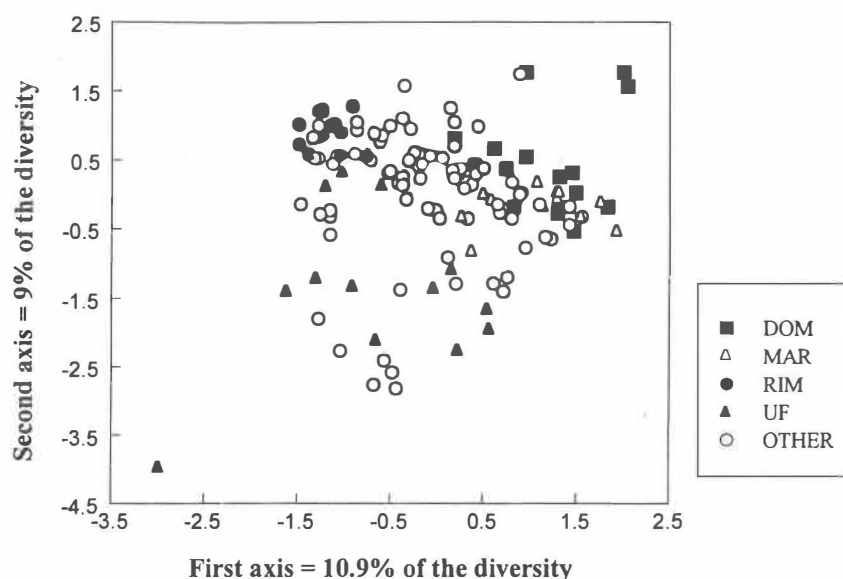
Population	Geographic origin	Sample size	H_i unbiased
DOM	Dominica	18	0.24
RIM	Mexico	14	0.09
SC	Colombia	9	0.11
TRD	Trinidad	15	0.41

Figure 1 shows the diversity existing within and among all of the Trinitario populations in the ICG,T analysed using RAPDs. The PCA, performed with the Statistica software (STATISTICA V5.1), reveals a separation into three different groups:

- the populations from Dominica (DOM) and Martinique (MAR)
- the UF population (Costa Rica)
- the RIM population (Mexico)

The accessions from the other populations (ICS, GS, CC, TRD) showed no clear separation and are referred to on the graph as "OTHER" populations. It may be noted additionally that two of the DOM accessions and one of the UF accessions appear to be clearly separated from all the other Trinitario accessions.

Figure 1. Plane defined by the first two axes of a Principal Component Analysis (PCA) performed on RAPD data from 141 Trinitario accessions.



Genetic diversity within some of the populations represented in the ICG,T

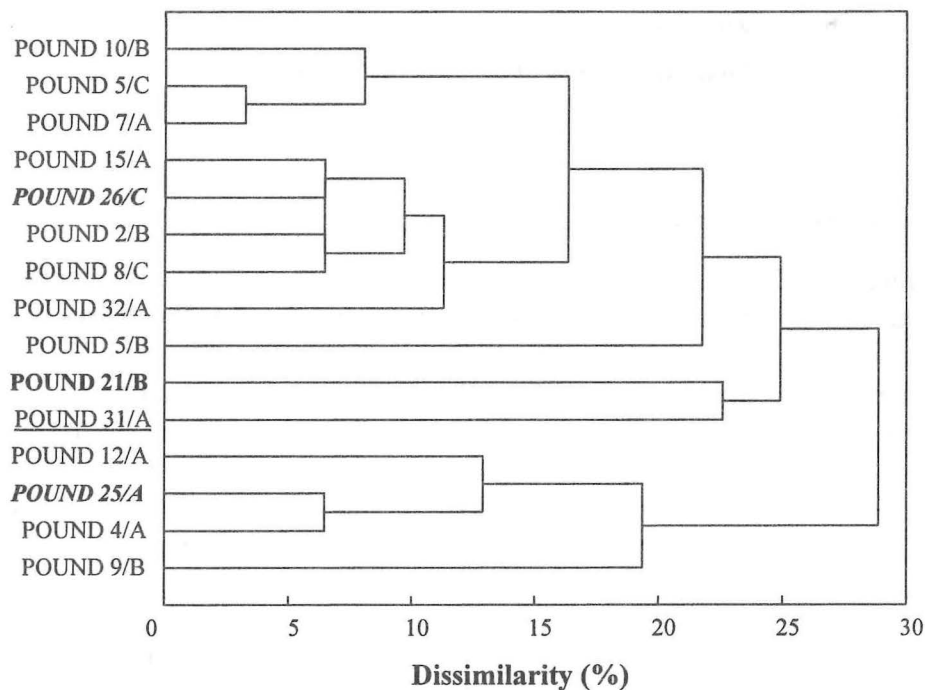
Ascending Hierarchical Classification analyses were performed on distances calculated from RAPD data. The distances between accessions were calculated as the degree of disagreement between their molecular profiles (proportion of different bands). The amalgamation method was the Unweighted Pair-Group Method using Arithmetic averages (UPGMA) which was performed on the RAPD data (% dissimilarity) to show the genetic relationship between accessions from the different populations of the ICG,T. Results for the POUND population (composed of the mother trees of the NA, PA, IMC, SCA and NAPO populations (Pound, 1943)), MO population and SCA population are presented here.

Figure 2 shows the genetic relationships between the POUND accessions. Accessions believed to be mother trees of the NA population are POUND 2/B, POUND 4/A, POUND 5/C, POUND 7/A, POUND 8/C, POUND 9/B, POUND 12/A and POUND 15/A (Pound, 1943) and are represented in plain text in Figure 2. It appears that some of these clones are closely related, such as POUND 5/C and POUND 7/A (3% dissimilarity), and POUND 2/B and POUND 8/C (6% dissimilarity). On the other hand, accessions such as POUND 9/B, POUND 4/A and POUND 12/A are found much less related to the other mother trees of the NA population. POUND 31/A (believed to be the mother tree of the SCA accessions (Pound 1943) and underlined in Figure 2) was found not to be closely related to any other POUND accession (between 23 and 48% dissimilarity). The same is true for POUND 21/B (believed to be the mother tree of the IMC accessions (Pound, 1943) and bold in Figure 2), which showed between 19 and 39% dissimilarity.

POUND 25/A and POUND 26/C (believed to be mother trees of the NAPO accessions (Pound, 1943) are not closely related (23% dissimilarity) but POUND 26/C is quite closely related to POUND 15/A (6% dissimilarity), which is one of the mother trees of the NA population.

It is interesting to note that POUND 5/B and POUND 5/C are found rather poorly related (23% dissimilarity). They are assumed to originate from budwood collected on two adjacent trunks growing on the same site (Bartley, 1993), which could be two seedlings originating from the same fruit. If this is the case, then the fruit probably originated from a cross between rather highly heterozygous parents.

Figure 2. Dendrogram depicting the genetic relationships among 15 accessions from the POUND population, obtained from a cluster analysis (UPGMA = Unweighted Pair-Group Method using Arithmetic averages) performed on % dissimilarity RAPD data.



Within the MO population, three major clusters were shown (Figure 3):

- MO 109
- MO 81, MO 3 and MO 9
- all the other MO accessions

These results support the hypothesis made by Bartley (1993) that MO accessions are not issued from one single mother tree, as suggested by Pound (1938). Bartley (1993), however, suggested that all the MO accessions with a high number should be MOQ

accessions (instead of MO), since only 80 MO trees were initially reported (Pound, 1938). This explanation of the MO origin is not supported by our findings since some accessions with high numbers, such as MO 121 and MO 129 are closely related to MO 14 (6% dissimilarity in both cases). On the other hand, accessions such as MO 3 and MO 9 are poorly related to MO 14, MO 20 and MO 76 (between 39 and 48% dissimilarity).

Figure 3. Dendrogram depicting the genetic relationships among 14 accessions from the MO population, obtained from a cluster analysis (UPGMA = Unweighted Pair-Group Method using Arithmetic averages) performed on % dissimilarity RAPD data.

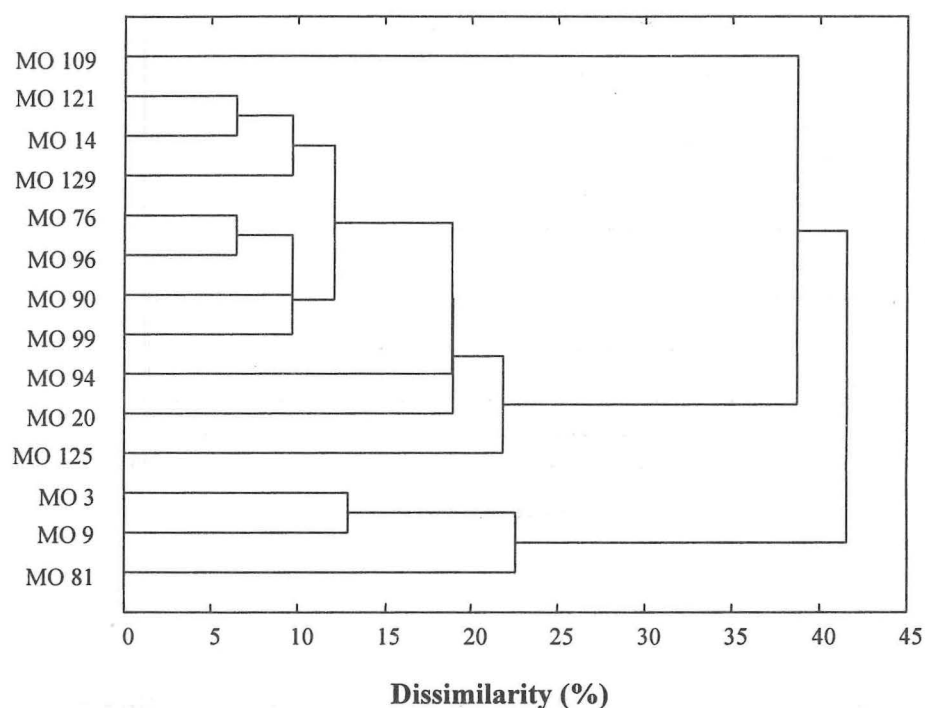
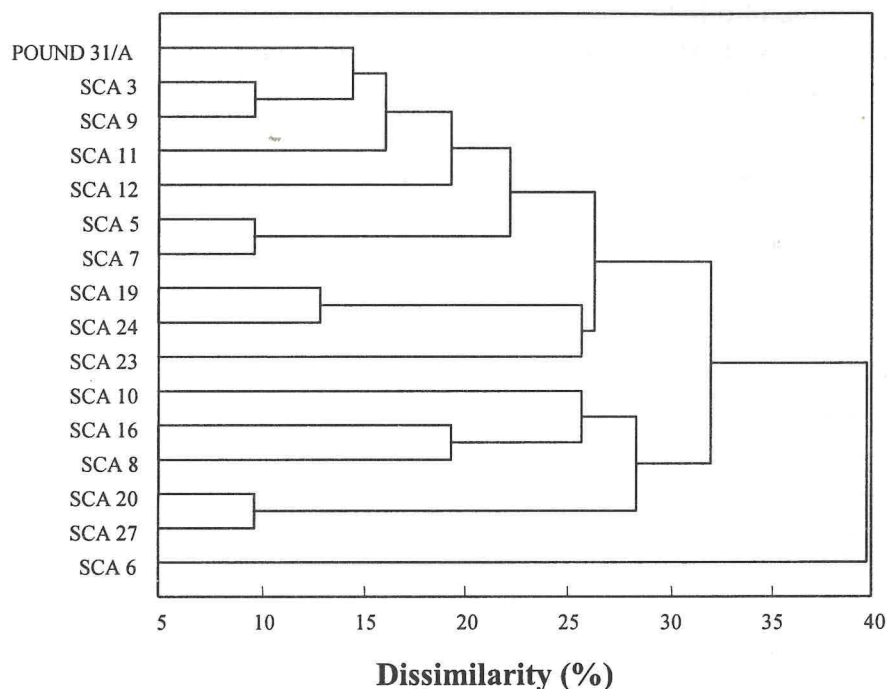


Figure 4 shows that SCA 6 is distinct from the other accessions in the SCA population (32 to 45% dissimilarity). This result suggests that the study made by Figueira (1997) to confirm the geographical origin of the SCA population by comparing the RAPD profile of SCA 6 with those obtained on cocoa accessions collected in 1987-89 on the banks of the Ucayali river should be extended by analysing additional SCA accessions. Some of these accessions such as SCA 3 and SCA 9 are found to be fairly closely related to POUND 31/A, (13 and 16% dissimilarity respectively). However, others such as SCA 6, SCA 7, SCA 27 and SCA 10 are rather poorly related to POUND 31/A (35, 29, 29 and 39% dissimilarity respectively). POUND 31/A originated from budwood collected on a tree adjacent to POUND 31, believed to be the mother tree from which all the SCA accessions originated (Pound, 1943). However, the results presented here do not support the hypothesis of a single mother tree origin for this population.

Figure 4. Dendrogram depicting the genetic relationships among 14 accessions from the SCA population, obtained from a cluster analysis (UPGMA = Unweighted Pair-Group Method using Arithmetic averages) performed on % dissimilarity RAPD data.



Conclusion

The results presented here permit a number of conclusions to be drawn. First, the collection of material in several estates in Trinidad carried out around ten years ago resulted in the capture of a rather high level of genetic diversity. In addition, this material appears to contain some interesting sources of resistance to *Phytophthora* (Iwaro, pers. com.). The material collected in Dominica (DOM) showed a lower level of genetic diversity but is of interest since some of these accessions present RAPD profiles quite different to the other Trinitario accessions. The material from Mexico (RIM) showed RAPD profiles very different to the accessions collected in Dominica and Martinique.

In some cases, the analysis of the genetic diversity between accessions from the same Upper Amazon populations gave data that do not support current hypotheses on the genetic origin of these populations. This is the case for the MO and SCA populations.

Future direction

The assessment of the genetic diversity existing in the ICG,T will continue, but we plan to use SSR molecular markers (Lanaud *et al.*, 1999) in 2001. This will provide more complete information, especially on the level of heterozygosity, due to the co-dominance of these markers.

References

- Bartley, B.G.D. (1993) Notes on the meaning and origins of clone names. Personal communication to M. End.
- Figueira, A. (1997) Was SCAVINA collected in the Ucayali River Basin? *INGENIC Newsletter* 3: 18-19.
- Pound, F.J. (1938) Cacao and Witches' Broom disease of South America with notes on other species of *Theobroma*. *Archives of Cocoa Research* 1: 26-71.
- Pound, F.J. (1943) Cacao and Witches' Broom disease. Report on a recent visit to the Amazon territory of Peru, September 1942 - February 1943. *Archives of Cocoa Research* 1: 73-91.
- Lanaud, C., Risterucci, A.M., Pieretti, I. , Falque, M., Bouet, A. and Lagoda, P.J.L (1999) Isolation and characterization of microsatellites in *Theobroma cacao* L. *Mol. Ecol.* 12 (8): 2141.