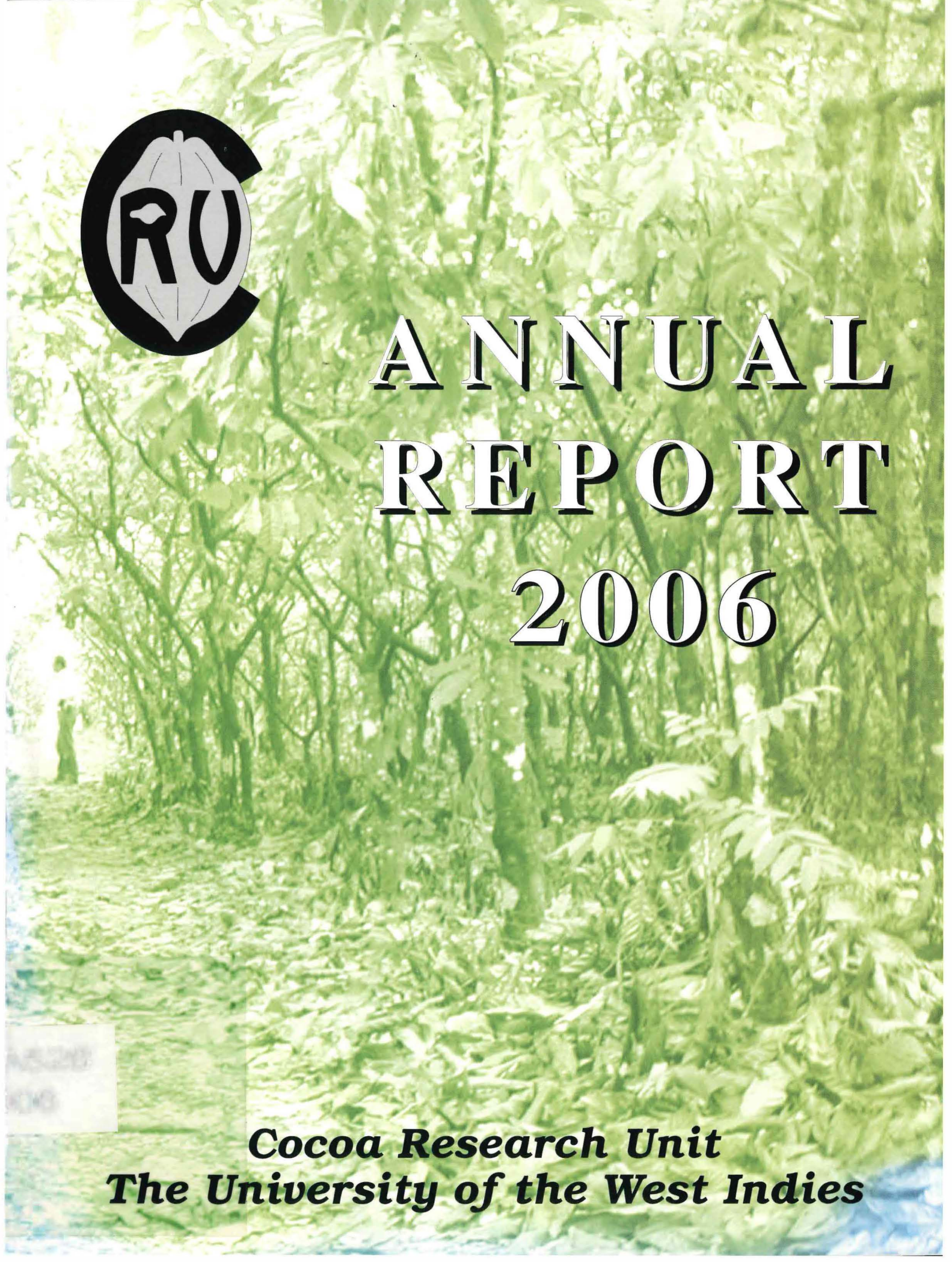

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Cover photograph. Intensively planted cocoa before (back cover) and after (front cover) pruning.

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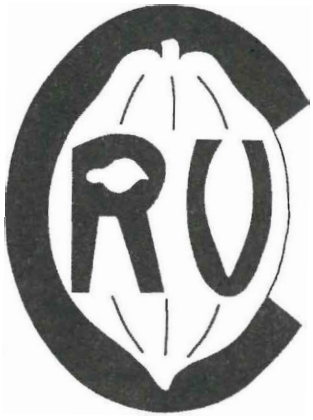

Cocoa Research Unit
The University of the West Indies
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2007

The contribution of the collaborative USDA/CRU project to resolve identity issues for trees in Marper Farm with missing labels

M. Boccara and D. Zhang

Introduction

Two fields in Marper Farm (Blocks C and D) were established by F.J. Pound following his expeditions to the upper Amazon between 1937 and 1942. After establishment, a survey was conducted in 1943 to check surviving trees and the infection rate of Witches' Broom disease. As a general rule, 2 replicate trees were planted in contiguous rows, one was discarded after assessment and a location number was given to the remaining one.

However, according to the 1943 records, some tree labels were already missing, and others were subsequently lost. When leaf samples were collected for DNA extraction in the USDA/CRU project, trees with no labels were given "MARPER" names.

Currently, in Marper Farm, 20 trees labelled MARPER are still alive in Block C, and 31 in block D; despite the lack of information about their identity, these trees are being replicated in the LNV Project to Safeguard the ICG,T (Table 1).

A main goal of the international collaborative project on DNA fingerprinting of Cocoa germplasm, which was started in 2001, was to confirm the identity of all trees in the ICG,T, and this led to renewed interest in the "MARPER" clones.

Achievements

Since 2001, leaves have been collected from every live tree in Blocks C and D of Marper Farm, in addition to other original accessions.

DNA samples were sent to the USDA-ARS Beltsville laboratory to be analysed with 15 recommended SSR primers, following the recommended protocol and guidelines (Saunders, 2000).

Data analysis

The results of the DNA profiles from USDA-ARS Beltsville laboratory are available for 1,400 clones from CRU, including 49 "MARPER" clones and have been used for different purposes:

- To identify the individual trees
- To place trees within appropriate accession groups.

Table 1. List of "MARPER" accessions and their locations.

Accession	Field location	DNA sample number	Comments
MARPER 1	C372	fp2549	Same location number as SJ 1/42 [POU] dead
MARPER 2	C363A	fp2527	Extra tree next to LP 3/2 [POU] location C363
MARPER 3	C1011	fp2602	Same location number as B 6/11 [POU] dead
MARPER 4	C782	fp2546	Tree PA 288 [PER] missing after landslide in 1943
MARPER 5	C782A	fp2542	Extra tree next to MARPER 4
MARPER 6	C783	fp2544	Tree LP 1/51 [POU] missing after landslide in 1943
MARPER 7	C784	fp2545	Tree MOQ 2/18 missing after landslide in 1943
MARPER 8	C216	fp2525	Same location number as LX 15 dead
MARPER 9	C597	fp2566	Same location number as CL 9/12 dead
MARPER 10	C895	fp2539	Same location number as AM 2/88 [POU] dead
MARPER 11	C492	fp2529	Same location number as CL 13/32 dead
MARPER 12	C942	fp2540	Same location number as AM 2/84 [POU] dead
MARPER 13	C748	fp2575	Same location number as MOQ 6/70 dead
MARPER 14	C622	fp2552	Same location number as LP 1/6 [POU] dead
MARPER 15	C449	fp2028	Same location number as CL 9/47 dead
MARPER 16	D750A	fp667	Extra tree next to NA 540 location D 751 dead
MARPER 17	C660	fp2368	Same location number as JA 3/3 [POU] dead
MARPER 18	D11	fp394	Label missing in 1943
MARPER 19	D22	fp414	Label missing in 1943
MARPER 20	D47	fp419	Label missing in 1943
MARPER 21	D31	fp80	Label missing in 1943
MARPER 22	C475	fp2345	Same location number as AM 2/49 [POU] dead
MARPER 24	D119	fp77	Same location number as NA 120 dead
MARPER 25	D122	fp82	Same location number as NA 38 dead
MARPER 27	D491A	fp84	Extra tree between NA 251 and PA 169
MARPER 28	D647A	fp76	Extra tree between IMC67,IMC45 and NA 157
MARPER 29	D307	fp69	Label missing in 1943
MARPER 30	D212A	fp86	Same location number as JA 6/16 [POU] dead
MARPER 31	D208	fp75	Same location number as B 7/13 [POU] dead
MARPER 33	D166	fp78	Same location number as B 18/9 [POU] dead
MARPER 34	D559	fp150	Label missing in 1943
MARPER 35	D755	fp690	Label missing in 1943
MARPER 37	D661	fp281	Label missing in 1943
MARPER 38	D167	fp318	Same location number as NA 242 dead
MARPER 39	D168	fp321	Same location number as B 22/15 [POU] dead
MARPER 40	D680	fp85	Same location number as NA 151 dead
MARPER 41	D706	fp227	Label missing in 1943
MARPER 42	D713	fp320	Extra tree next to MO 4 location D 684 dead
MARPER 43	D747	fp263	Label missing in 1943
MARPER 44	D251	fp74	Same location number as B 14/14 [POU]
MARPER 45	D251A	fp83	Extra tree between NA 98 and SLC 24
MARPER 46	D764	fp251	Label missing in 1943
MARPER 47	C39	fp1270	Same location number as AM 1/38 [POU] dead
MARPER 48	D758A	fp244	Extra tree between PA 159 [PER] and B 14/17 [POU] dead
MARPER 50	D790B	fp674	Extra tree next to PA 151 [PER] location D 750 alive
MARPER 51	D800	fp668	Same location number as NA 345 dead
MARPER 52	D826A	fp711	Extra tree between NA 232 and NA 300 dead
MARPER 53	D776A	fp670	Same location number as NA 406 dead
MARPER 54	D777A	fp675	Same location number as NA 537 dead
MARPER 55	C249	fp1365	Same location number as MOQ 4/5 dead

Methods

Genetic grouping of the "MARPER" clones was assessed in relation to the 1,400 clones sampled in the ICG, T, using dissimilarity analysis (DARwin software, 5.0.142) and Principal Component Analysis (Genetix software, v.4.03).

The similarity of DNA profiles was examined and used in combination with all information available in historical records, publications and maps.

Results

Genetic diversity of the MARPER clones

The Principal component analysis (PCA) using the Genetix software (Figure 1) shows that:

- Some accessions labelled MARPER fall in the PA group
- Some accessions fall in the NA group
- Some accessions fall in the Refractario group
- Some belong to other genetic groups such as Trinitario
- Many accessions cannot be assigned to a distinctive group.

The Cluster analysis of the 49 "MARPER" DNA samples using the DARwin software and the detailed comparison of their multilocus profile provided additional information (Table 2).

Table 2. Assignments to groups of "MARPER" accessions.

Accessions clustered with PA accessions						
MARPER 4	fp2546	C782		MARPER 5	fp2542	C782A
MARPER 27	fp84	D491A		MARPER 42	fp320	D713

Accessions clustered with Refractarios accessions						
MARPER 11	fp2529	C492		MARPER 12	fp2540	C942
MARPER 19	fp414	D22		MARPER 20	fp419	D47
MARPER 21	fp80	D31		MARPER 43	fp263	D747

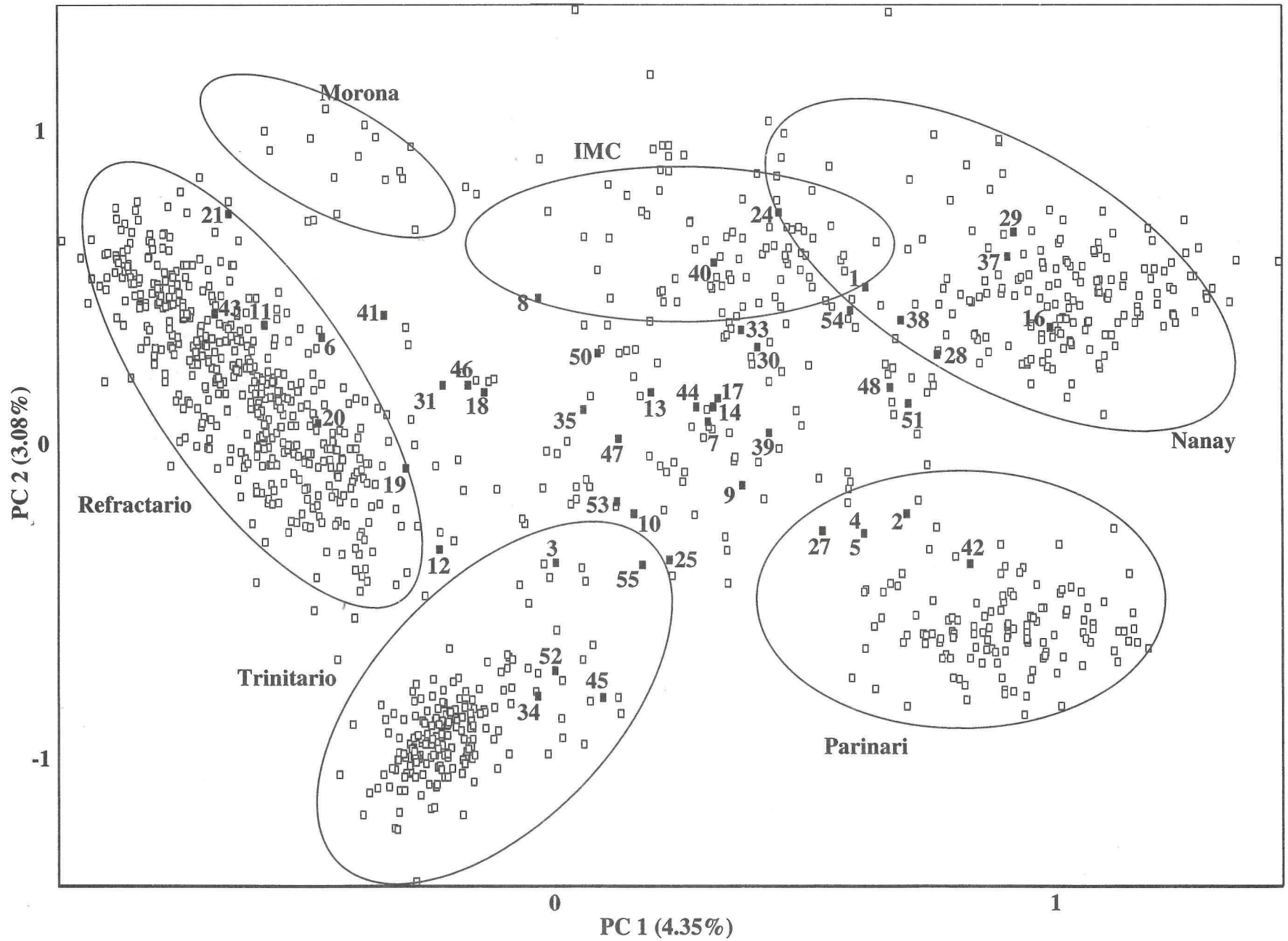
Accessions clustered with NA accessions						
MARPER 16	fp613	D750A		MARPER 28	fp76	D647A
MARPER 29	fp69	D307		MARPER 37	fp281	D661
MARPER 38	fp318	D167				

Accessions clustered with Trinitarios accessions						
MARPER 3	fp2602	C1011		MARPER 25	fp82	D122
MARPER 34	fp150	D559		MARPER 35	fp690	D755
MARPER 45	fp83	D251A		MARPER 47	fp1270	C39
MARPER 52	fp711	D826A		MARPER 55	fp1365	C249

Trees showing a PA profile

MARPER 4 location C782 and MARPER 5 location C782A share the same profile and belong to the PA group; the original tree PA 288 [PER] planted in that position went missing after a

Figure 1. Principal component analysis for 1,400 accessions from the ICG,T. Trees with a MARPER label are shown as solid points.



landslide: these two trees could have re-grown from it.

MARPER 27 location D491A is an extra tree growing next to two PA accessions which are still alive (PA 189 [PER], PA 169 [PER]) and three PA accessions now dead (PA 127 [PER], PA 157 [PER] and PA 202 [PER]), the latter two accessions having been duplicated in UCRS. Profile comparisons show that MARPER 27 is not a duplicate of any of its neighbours, but it belongs to the PA group. It could be a seedling of one of them or even the missing clone PA 127 [PER].

MARPER 42 location D713 shows a PA profile and matches for 14/15 markers the profiles of MO 4 and IMC 41. We suggested in the last annual report (Boccara *et al.*, 2005) that these identical trees could have been propagated from a PA accession seedling instead of from their mother-trees now dead: MARPER 42 could be that seedling.

Trees showing a NA profile

The accession labelled MARPER 16 location D750A is growing where NA 540 (now dead) was planted; molecular analysis concerning the duplicate NA 540 tree in field 5B shows a Trinitario profile, indicative of propagation mistakenly done from the rootstock. MARPER 16 is probably the real NA 540 or could be a seedling from that tree.

MARPER 28 location D647A, and two of its neighbours IMC 67 location D647 and IMC 45 location D648 show NA profiles. All these trees, originally planted on a very steep terrain and now lying on the ground, could be regrowths or seedlings originating from NA 157 location D649. The replicate trees of NA 157 in UCRS should be checked.

The labels of trees planted in D307 and D661 were missing in 1943, and the names MARPER 29 and MARPER 37 have been assigned to them. DNA analysis shows that these trees belong to the NA group.

MARPER 38 is a tree growing at the position D167 and was tentatively renamed NA 242 from the 1943 records. This result confirms that it is in the NA group and it is likely to be NA 242.

Trees showing a Refractario profile

MARPER 11, fp2529 location C492 is growing where, according to the 1943 records, CL13/32 was originally planted. Since it shows a Refractario profile, it is probably CL13/32. This is also the case of MARPER 12 location D942; it was a neighbour of AM 2/84 [POU] now missing.

The names MARPER 19, MARPER 20, MARPER 43 were given to trees without labels planted in D22, D47 and D747 respectively: the DNA profile analysis shows that all 3 trees belong to the Refractario group.

The profile of the tree MARPER 21 growing in D31 matches perfectly its neighbour, B 7/3 [POU] in D30: they are duplicate trees. Their similar morphology had previously been noted.

Trees showing a Trinitario profile

The dissimilarity analysis shows that eight MARPER accessions are closely related and all show a Trinitario profile, implying that they are rootstock. (Figure 2).

MARPER 3, MARPER 25, MARPER 47, MARPER 55 occupy locations where the original accession has disappeared, whilst MARPER 45 and MARPER 52 are extra trees. MARPER 34 and 35 had lost their identification labels in 1943.

Trees not falling in any group

Even if the results of DNA analysis did not place with certainty the remaining MARPER clones in

Figure 2. Trees showing a Trinitario profile; excerpt of the dendrogram of dissimilarity run on 1,400 DNA samples from cacao accessions in the ICG,T.

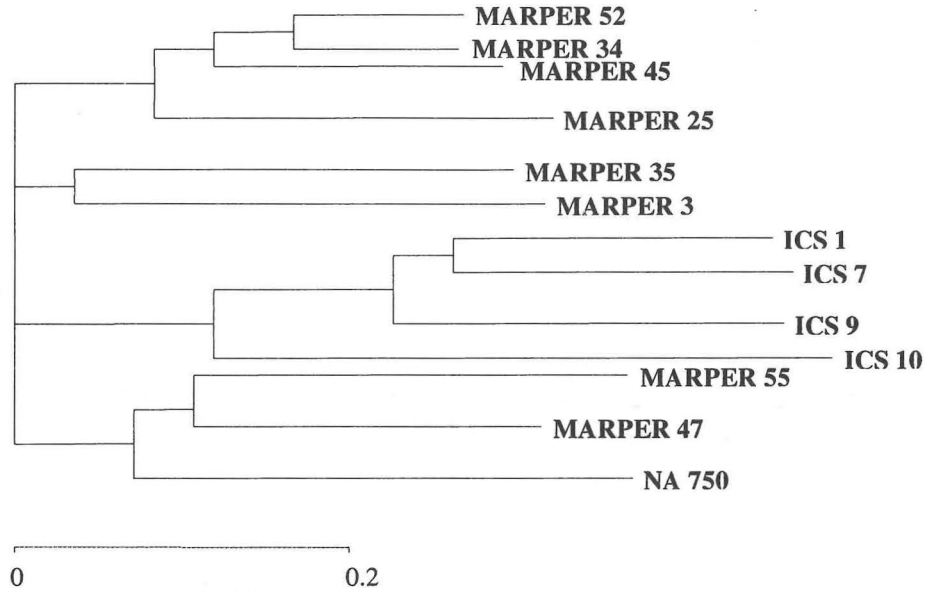
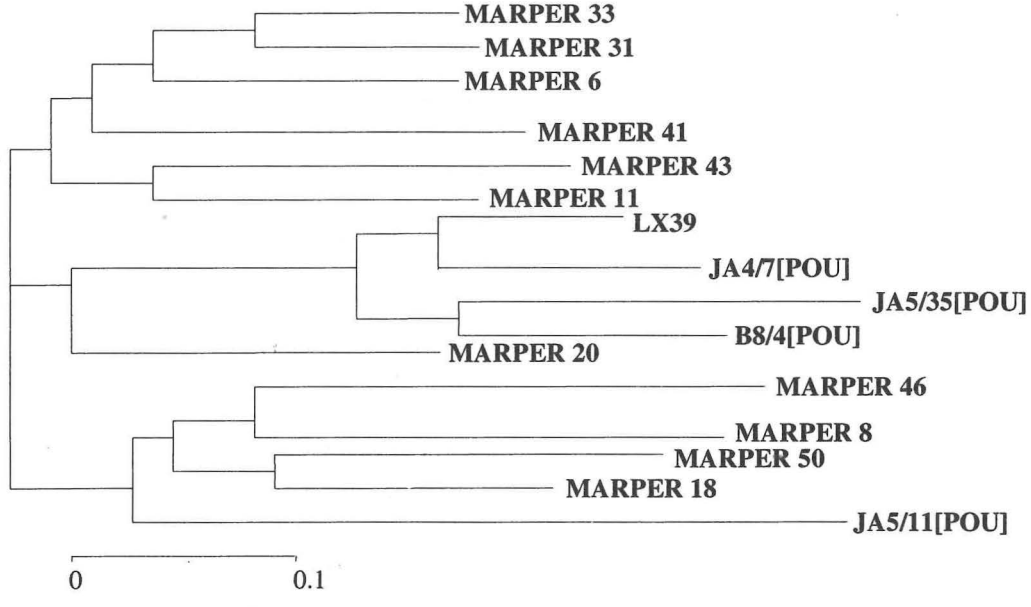


Figure 3. Trees showing a Refractario-like profile; excerpt of the dendrogram of dissimilarity run on 1,400 DNA samples from cacao accessions in the ICG,T.



a predefined group, the dendrogram constructed with the DARwin software (Figure 3) shows eight accessions in the same cluster as Refractarios (Table 3). Phenotypic diversity analysis could be very valuable for further assessment.

According to the PCA the remaining "MARPER" trees (Table 4) do not cluster clearly in any predefined group; however, the dendrogram constructed with the DARwin software shows that they are closely related (Figure 4).

Table 3. "MARPER" accessions possibly belonging to the Refractario group.

Accession	Field location	DNA sample number
MARPER 6	C783	fp2544
MARPER 8	C216	fp2525
MARPER 11	C492	fp2529
MARPER 18	D11	fp394
MARPER 31	D208	fp75
MARPER 33	D166	fp78
MARPER 41	D706	fp227
MARPER 46	D764	fp251
MARPER 50	D790B	fp674

Figure 4. Trees not falling into any group; excerpt of the dendrogram of dissimilarity run on 1,400 DNA samples from cacao accessions in the ICG,T.

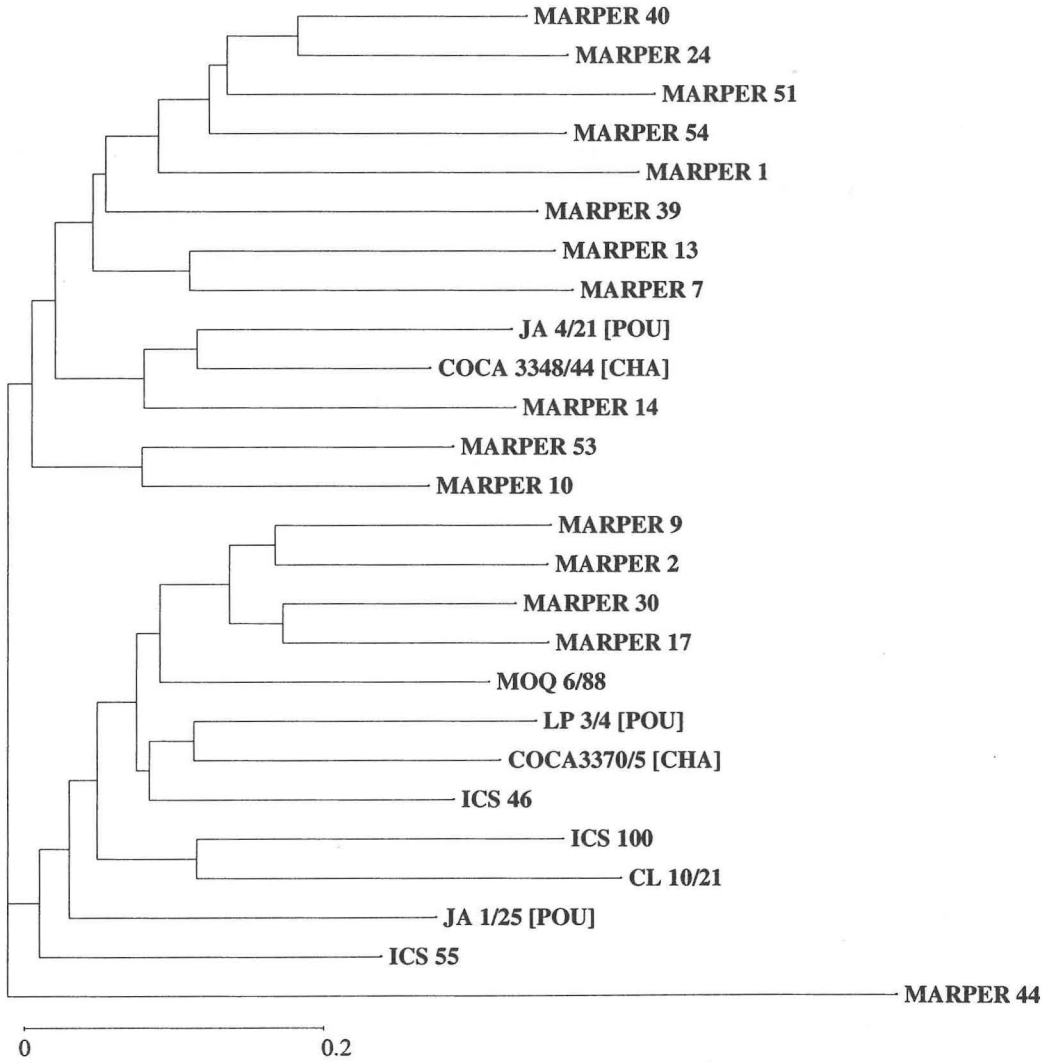


Table 4. "MARPER" accessions not assigned to a predefined group.

Accession	Field location	DNA sample number
MARPER 1	C372	fp2549
MARPER 2	C363A	fp2527
MARPER 7	C784	fp2545
MARPER 9	C597	fp2566
MARPER 10	C895	fp2539
MARPER 13	C748	fp2575
MARPER 14	C622	fp2552
MARPER 17	C660	fp2368

Accession	Field location	DNA sample number
MARPER 24	D119	fp77
MARPER 30	D212A	fp86
MARPER 39	D168	fp321
MARPER 40	D680	fp85
MARPER 44	D251	fp74
MARPER 51	D800	fp668
MARPER 53	D776A	fp670
MARPER 54	D777A	fp675

It is noteworthy that each of these trees, without exception, is occupying a location where the original accession is reported dead. One explanation could be that they are non-Trinitario surviving rootstock, or spontaneous hybrids from upper Amazon or Refractario accessions.

We found out that the clone MARPER 44, erroneously renamed B14/14 [POU] to match the old records, does not belong to the Refractario group: its MARPER name must be reinstated.

Discussion and conclusion

The use of 15 markers has been efficient in completing the unambiguous identification of accessions unlabelled 60 years ago. While more than one half of the "MARPER" clones can be assigned to an accession group, others were shown to be Trinitario rootstock, and the remaining clones may be hybrids or non-Trinitario rootstocks.

After assessment, clones of interest should be duplicated and transferred to UCRS for safe conservation.

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