











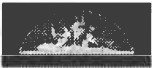







ANNUAL REPORT 2005

***Cocoa Research Unit
The University of the West Indies***

Annual Report 2005. St. Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies. 83 pp.

The work of CRU is made possible by support from

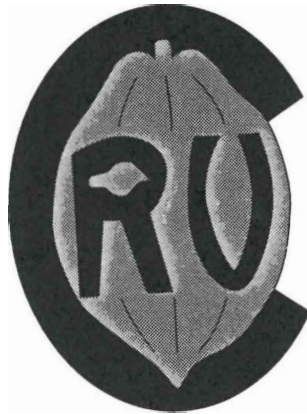
	World Cocoa Foundation, USA		Ministry of Agriculture, Land and Marine Resources (MALMR), Government of the Republic of Trinidad and Tobago
	The Biscuit, Cake, Chocolate & Confectionary Association (BCCCA), UK		Masterfoods, UK
	Cadbury Ltd., UK		United States Department of Agriculture, USA
	United Nations Common Fund for Commodities (CFC)		The University of Hamburg, Germany
	Centre de coopération internationale en recherche agronomique pour le développement (CIRAD), France		The University of Reading, UK
	Guittard Chocolate Company, Burlingame, USA		The University of the West Indies (UWI), Trinidad and Tobago and Barbados
	International Cocoa Germplasm Database (ICGD)		Forschungskreis der Ernährungsindustrie e.V.
	Lindt & Sprüngli (International) AG, Switzerland		Arbeitsgemeinschaft industrieller Forschungsvereinigungen "Otto-von-Guericke" e.V.

Cocoa Research Unit
The University of the West Indies
St. Augustine, Trinidad and Tobago

Tel. +1 868 662 8788
+1 868 662 2002 Ext. 2115
Fax +1 868 662 8788
E-mail cru@cablenett.net

Cover photograph. Cacao seedlings raised in a hydroponic system for micrografting at EEN San Carlos, Ecuador.

Annual Report 2005



**Cocoa Research Unit
The University of the West Indies
St. Augustine, Trinidad and Tobago
2006**

Progress in resolving identity issues among the Parinari accessions held in Trinidad: the contribution of the collaborative USDA/CRU project

M. Boccara and D. Zhang

Introduction

While searching for trees free of Witches' Broom disease in 1938, Dr. F.J. Pound observed about twenty of them on the left bank of the Marañon River above Parinari in Peru. He collected pods from these trees and seeds were planted in Barbados. After a suitable quarantine period, healthy budwood from the seedlings was forwarded to Trinidad, budded onto rootstock and subsequently planted, mostly in Marper Farm with some on the ICTA campus (now UWI). There is no clear indication about the number of trees selected, pods collected and seeds planted, however records have been kept of the identity of the established 'clones'.

Although 277 Parinari clones are reported to have been introduced to Trinidad (Pound, 1943), records available in CRU show that in 1943 only 147 clones were present in Marper Farm: 136 in Block D and 11 in Block C. Other PA trees were planted on the ICTA campus, among them 2 clones (PA7 [PER] and PA 35 [PER], Bartley pers. comm.) not represented in Marper.

When the ICG,T was established from 1986 to 1994, some PA clones were already missing. Only 113 clones were available, and 16 trees of each of these were planted per plot in UCRS.

Currently, in Marper Farm, 92 trees labelled PA are still alive in Block D, 9 in Block C and in UCRS, 111 clones are replicated in 133 plots (Table 1).

An international collaborative project on DNA fingerprinting of cacao germplasm was started in 2001, and priority was given to the analysis of Upper Amazon material such as the 'Parinaris' since they are of special interest to the international cocoa community.

Achievements

Leaves have been collected from every live tree in Blocks C and D of Marper Farm and from trees in UCRS when absent in Marper. Collection of some extra leaf samples was also undertaken for verification purposes.

A total of 158 samples were collected, including 101 from original trees from Marper fields, 16 from UCRS trees now absent from Marper as well as 41 samples from replicated trees in UCRS to check their conformity.

DNA samples were sent to the USDA-ARS¹ Beltsville laboratory to be analysed with 15 selected SSR primers, following a recommended protocol and guide-lines (Saunders, 2000).

Data analysis

The results of the DNA profiles from USDA-ARS Beltsville laboratory are available for 1,200 clones from the ICG,T, including the PA accessions and have been used for different purposes:

¹ United States Department of Agriculture – Agriculture Research Service

Table 1. List of PA accessions and their locations in 2005.

	Marper Farm		UCRS			Marper Farm		UCRS	
PA 1 [PER]	D 111	+	5B		PA 81 [PER]	D 675	†	4A	+
PA 2 [PER]	D 101	+	5A		PA 82 [PER]	D 666	+	5B	
PA 3 [PER]	D 571	+	--		PA 84 [PER]	D 264	+	5B	+
PA 4 [PER]	C 1108	+	5B		PA 88 [PER]	D 677	+	5B	+
PA 5 [PER]	C 1101	+	--		PA 90 [PER]	D 627	+	5B	
PA 7 [PER]	**	†	--		PA 95 [PER]	D 663	+	5B	+
PA 12 [PER]	D 54	+	5A	+	PA 98 [PER]	D 295	+	--	
PA 13 [PER]	D158/D159	++	5A, 6B		PA 101 [PER]	D 831	†	--	
PA 14 [PER]	D 115	†	--		PA 103 [PER]	D 293	†	--	
PA 15 [PER]	D 596	+	6B		PA 105 [PER]	D 285	+	4A, 5A	+
PA 16 [PER]	D 573	+	6B	+	PA 107 [PER]	D 284	+	5A	
PA 18 [PER]	D 55	+	5A, 6B	+	PA 109 [PER]	D 304	†	6B	
PA 20 [PER]	D 24	+	4A		PA 110 [PER]	D 805	†	--	
PA 24 [PER]	D 401	+	5B	+	PA 111 [PER]	D 783	†	--	
PA 25 [PER]	D 262	†	--		PA 113 [PER]	D 384	+	5A, 5B	+
PA 26 [PER]	D 672	†	--		PA 114 [PER]	D 837	†	5B	+
PA 27 [PER]	D 676	+	5B	+	PA 115 [PER]	D 324	+	5B	
PA 29 [PER]	D 652	+	5B		PA 117 [PER]	D 332	†	--	
PA 30 [PER]	D 266	†	6B	+	PA 118 [PER]	D 288	+	5B	
PA 31 [PER]	D 670	†	--		PA 120 [PER]	D 318	+	6B	+
PA 32 [PER]	D 253	+	5A		PA 121 [PER]	D 393	+	6B	+
PA 33 [PER]	D 257	†	--		PA 123 [PER]	D 462	+	5A	
PA 34 [PER]	D 259	+	5B	+	PA 124 [PER]	D 492	+	4A, 6B	+
PA 35 [PER]	**	†	6B	+	PA 125 [PER]	D 433	+	4A, 5B	
PA 37 [PER]	D 244	+	5A	+	PA 126 [PER]	D 247	+	6B	+
PA 39 [PER]	D 685	†	5A	+	PA 127 [PER]	D 488	†	--	
PA 41 [PER]	D 667	+	6B		PA 128 [PER]	D 443	+	5A	+
PA 42 [PER]	D 643	†	--		PA 132 [PER]	D 357	+	5A	
PA 44 [PER]	D 233	+	6B		PA 134 [PER]	D 442	+	4A, 5A	
PA 45 [PER]	D 261	+	4A, 5A	+	PA 135 [PER]	D 481	+	4A, 5A	
PA 46 [PER]	D 235	+	6B	+	PA 136 [PER]	D 455	+	5A, 5B	
PA 48 [PER]	D 234	†	5A	+	PA 137 [PER]	D 430	+	5A	+
PA 49 [PER]	C 586	+	5A, 5B		PA 138 [PER]	D 842	†	--	
PA 51 [PER]	D 260	+	6B		PA 139 [PER]	D 426	+	4A, 5A	+
PA 52 [PER]	D 654	†	5A	+	PA 140 [PER]	D 439	+	5A	
PA 53 [PER]	D 243	+	5A		PA 141 [PER]	D 463	+	5B	+
PA 56 [PER]	D 238	+	5B		PA 143 [PER]	C 985	†	5A	+
PA 58 [PER]	D 678	†	--		PA 146 [PER]	D 441	†	--	
PA 59 [PER]	D 256	†	--		PA 148 [PER]	D 423	†	--	
PA 61 [PER]	D 121	+	6B		PA 149 [PER]	D 810	+	5B	+
PA 62 [PER]	D 255	+	--		PA 150 [PER]	D 679	+	6B	
PA 63 [PER]	D 348	+	5A	+	PA 151 [PER]	D 790	+	5B	
PA 64 [PER]	D 263	†	--		PA 152 [PER]	D 700	†	--	
PA 65 [PER]	D 507	+	5B	+	PA 156 [PER]	D 447	†	5A	+
PA 66 [PER]	C 880	+	5B		PA 157 [PER]	D 452	†	5B	+
PA 67 [PER]	D 629	+	5A, 5B	+	PA 159 [PER]	D 756	†	--	
PA 68 [PER]	D 638	†	5B	+	PA 165 [PER]	D 714	+	5B	+
PA 70 [PER]	D 634	+	5B	+	PA 167 [PER]	D 736	+	--	
PA 71 [PER]	D 674	†	6B	+	PA 168 [PER]	D 479	+	5A	
PA 72 [PER]	D 633	+	4A, 5B		PA 169 [PER]	D 491	+	6B	
PA 73 [PER]	D 254	+	--		PA 171 [PER]	D 467	+	5A, 6B	

+ Tree(s) alive and DNA sampled

-- No record

† Tree dead

** Trees not planted in Marper, but were on the ICTA/UWI campus

Table 1 (continued). List of PA accessions and their locations in 2005.

	Marper Farm		UCRS		Marper Farm		UCRS	
PA 172 [PER]	D 728	†	--		PA 271 [PER]	C 716	+	5A
PA 173 [PER]	D 851	†	5B	+	PA 272 [PER]	D 37	+	5A
PA 175 [PER]	D 738	+	5B	+	PA 275 [PER]	C 764	+	4A
PA 176 [PER]	D 704	+	4A, 5A		PA 276 [PER]	D 520	†	--
PA 179 [PER]	D 472	+	5B		PA 279 [PER]	D 59	+	6B
PA 181 [PER]	D 460	†	--		PA 281 [PER]	D 28	†	--
PA 184 [PER]	D 723	+	5B		PA 285 [PER]	D 378	+	--
PA 185 [PER]	D 735	+	5B		PA 288 [PER]	C 782	†	--
PA 186 [PER]	D 446	-	--		PA 289 [PER]	C 803	+	4A, 5B
PA 187 [PER]	D 482/D737	++	4A, 5B		PA 291 [PER]	D 214	+	6B
PA 188 [PER]	D 724	+	5B	+	PA 293 [PER]	C 817	+	--
PA 189 [PER]	D 489	+	4A, 5B		PA 293 [PER]	D 762	+	4A, 5A
PA 191 [PER]	D 743	+	5B	+	PA 294 [PER]	D 330	+	5B
PA 194 [PER]	D 707	†	4A, 5B	+	PA 295 [PER]	D 371	†	--
PA 195 [PER]	D 493	†	6B	+	PA 296 [PER]	D 495	+	6B
PA 196 [PER]	D 458	+	5B	+	PA 297 [PER]	--	†	6B
PA 200 [PER]	D 710	+	4A, 5B	+	PA 299 [PER]	C 936	+	5B
PA 202 [PER]	D 453	†	5A	+	PA 300 [PER]	D 544	+	5B
PA 203 [PER]	D 709	+	--		PA 301 [PER]	D 733	+	5A
PA 205 [PER]	D 715	+	5B		PA 303 [PER]	D 500	+	6B
PA 206 [PER]	D 745	†	--		PA 310 [PER]	D 732	†	5A
PA 207 [PER]	D 731	+	4A		PA 312 [PER]	--	†	6B
PA 211 [PER]	D 766	+	5B		PA 319 [PER]	D 554	†	--
PA 218 [PER]	D 708	+	6B		PA 320 [PER]	D 721	†	--

+ Tree(s) alive and DNA sampled

-- No record

† Tree dead

** Trees not planted in Marper, but were on the ICTA/UWI campus

- To assess the population identity of the Parinari group
- To discover potential mislabelling and to find conceivable explanations
- To detect off-type clones in the accession group
- To verify that the duplicate trees are identical
- To place individual trees within appropriate accession groups
- To assess population admixture.

Methods

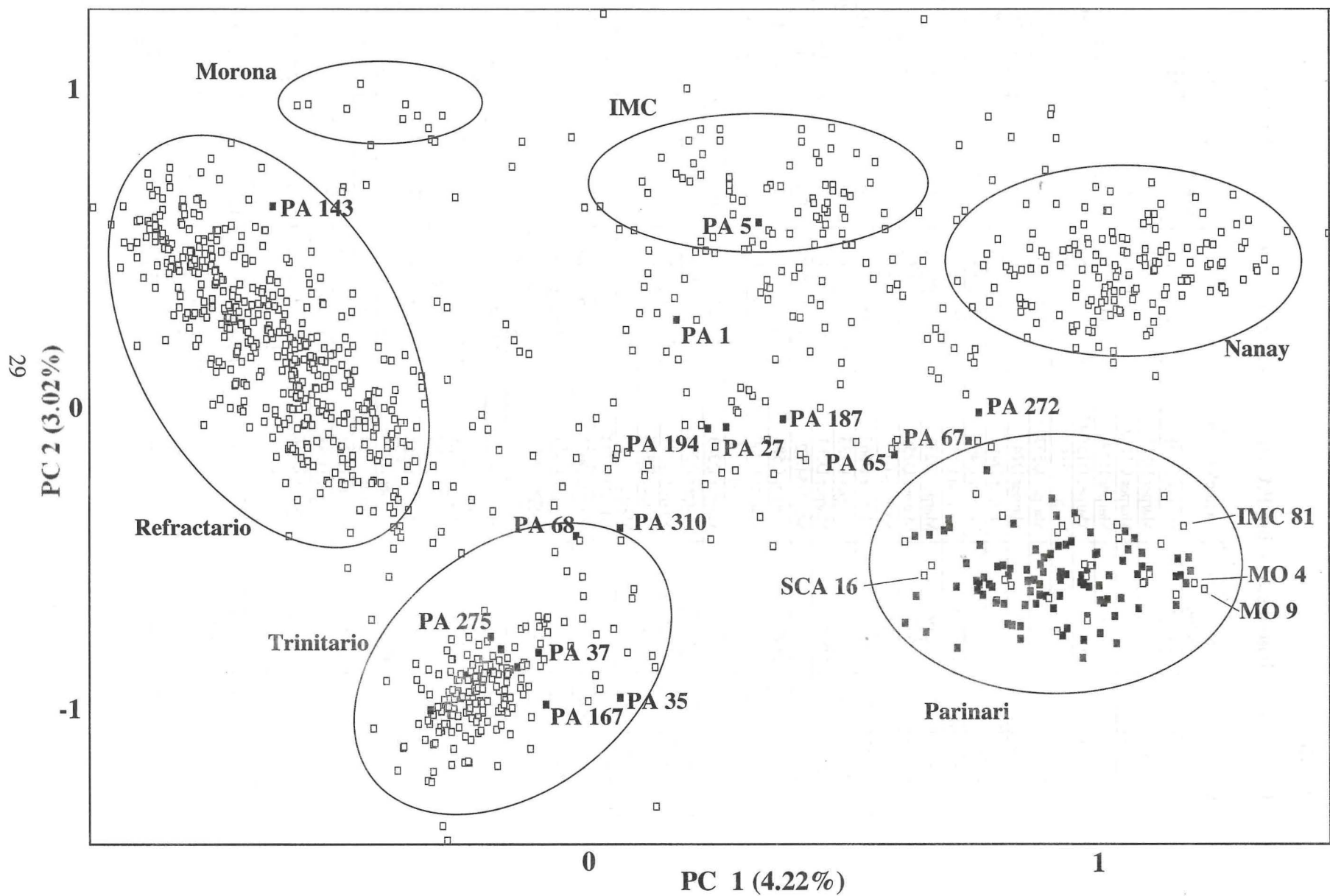
- Genetic diversity of the 132 PA clones was assessed in relation to the 1,200 clones sampled in the ICG,T, using dissimilarity analysis (DARwin software, 5.0.142) and principal component analysis (PCA)(Genetix software, v.4.03).
- Duplicate trees were assessed by identifying matching multilocus genotypes among PA accessions.
- Mislabelled trees were identified by comparing their multilocus profile to the reference tree or a putative replicate.
- The identities of off-types were sought from matching profiles, and by using all the information available in historical records, publications and maps.

Results

Table 2. Confirmed surviving PA accessions at Marper Farm and UCRS.

Accession	DNA sample number	Field plot location	Accession	DNA sample number	Field plot location
PA 2 [PER]	FP1114	Marper D101	PA 134 [PER]	FP1197	Marper D442
PA 3 [PER]	FP184	Marper D571	PA 135 [PER]	FP392	Marper D481
PA 4 [PER]	FP619	Marper C1108	PA 136 [PER]	FP147	Marper D430
PA 12 [PER]	FP424	Marper D 54	PA 137 [PER]	FP307	Marper D430
PA 13 [PER]	FP821	Marper D158/159	PA 139 [PER]	FP308	Marper D426
PA 15 [PER]	FP177	Marper D596	PA 140 [PER]	FP1201	Marper D439
PA 16 [PER]	FP189	Marper D573	PA 141 [PER]	FP1249	Marper D463
PA 20 [PER]	FP410	Marper D24	PA 149 [PER]	FP252	Marper D810
PA 24 [PER]	FP384	Marper D401	PA 150 [PER]	FP144	Marper D679
PA 29 [PER]	FP161	Marper D401	PA 151 [PER]	FP114	Marper D790
PA 30 [PER]	FP1634	5B C144 T1	PA 156 [PER]	FP1396	5A D295 T1
PA 32 [PER]	FP219	Marper D253	PA 157 [PER]	FP17	5B F466 T3
PA 34 [PER]	FP333	Marper D259	PA 165 [PER]	FP239	Marper D714
PA 39 [PER]	FP634	5A D264 T1	PA 168 [PER]	FP391	Marper D479
PA 41 [PER]	FP277	Marper D667	PA 169 [PER]	FP118	Marper D491
PA 44 [PER]	FP1141	Marper D233	PA 171 [PER]	FP149	Marper D467
PA 45 [PER]	FP67	Marper D261	PA 173 [PER]	FP13	5B F480 T8
PA 48 [PER]	FP1959	5A D354 T2	PA 175 [PER]	FP266	Marper D738
PA 49 [PER]	FP88	Marper C586	PA 176 [PER]	FP234	Marper D704
PA 51 [PER]	FP212	Marper D260	PA 179 [PER]	FP376	Marper D472
PA 52 [PER]	FP1399	5A D310 T8	PA 184 [PER]	FP229	Marper D723
PA 53 [PER]	FP1169	Marper D243	PA 185 [PER]	FP268	Marper D735
PA 56 [PER]	FP1162	Marper D238	PA 186 [PER]	FP378	Marper D446
PA 61 [PER]	FP208	Marper D121	PA 187 [PER]	FP228	Marper D737
PA 63 [PER]	FP1202	Marper D348	PA 188 [PER]	FP238	Marper D724
PA 70 [PER]	FP278	Marper D634	PA 189 [PER]	FP137	MarperD489
PA 71 [PER]	FP2464	6B D189 T14	PA 191 [PER]	FP265	Marper D743
PA 72 [PER]	FP305	Marper D633	PA 196 [PER]	FP176	Marper D458
PA 73 [PER]	FP325	Marper D254	PA 200 [PER]	FP111	Marper D710
PA 82 [PER]	FP279	Marper D666	PA 202 [PER]	FP1397	5A D309 T1
PA 84 [PER]	FP334	Marper D264	PA 203 [PER]	FP106	Marper D709
PA 88 [PER]	FP294	Marper D677	PA 207 [PER]	FP133	Marper D731
PA 90 [PER]	FP154	Marper D627	PA 211 [PER]	FP248	Marper D766
PA 95 [PER]	FP280	Marper D663	PA 218 [PER]	FP113	Marper D708
PA 98 [PER]	FP205	Marper D295	PA 271 [PER]	FP551	Marper C716
PA 105 [PER]	FP1179	Marper D285	PA 275 [PER]	FP560	Marper C764
PA 107 [PER]	FP1168	Marper D284	PA 279 [PER]	FP426	Marper D59
PA 113 [PER]	FP306	Marper D384	PA 289 [PER]	FP559	Marper C803
PA 115 [PER]	FP1196	Marper D324	PA 291 [PER]	FP50	Marper D214
PA 118 [PER]	FP1180	Marper D288	PA 293 [PER]	FP258	Marper D762
PA 120 [PER]	FP194	Marper D318	PA 294 [PER]	FP1203	Marper D330
PA 121 [PER]	FP1185	Marper D393	PA 296 [PER]	FP162	Marper D495
PA 123 [PER]	FP160	Marper D462	PA 297 [PER]	FP2421	6B D208 T1
PA 124 [PER]	FP1251	Marper D492	PA 299 [PER]	FP571	Marper C936
PA 125 [PER]	FP1200	Marper D433	PA 300 [PER]	FP382	Marper D544
PA 126 [PER]	FP331	Marper D247	PA 301 [PER]	FP270	Marper D733
PA 128 [PER]	FP388	Marper D443	PA 303 [PER]	FP185	Marper D500
PA 132 [PER]	FP389	Marper D357	PA 310 [PER]	FP1958	5A D288 T2

Figure 1. Principal component analysis for 1,200 accessions from the ICG,T. Trees with a PA label are shown as solid points.



Genetic diversity of the PA clones and potential mislabelling

The PCA using the Genetix software (Figure 1) shows clearly that the PA accessions form a group that is distinct from the rest of the clones analysed.

It also shows that:

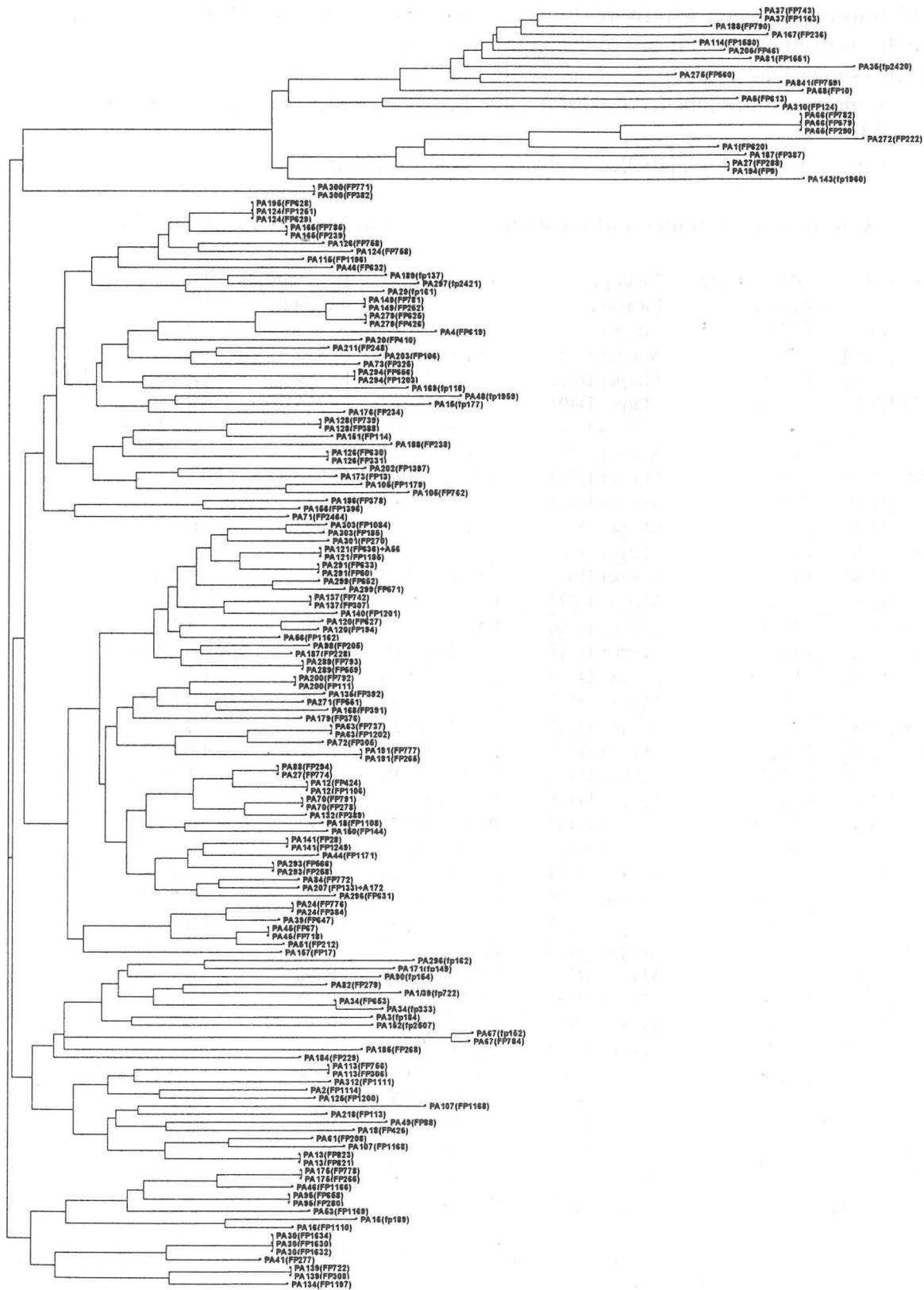
- Some accessions labelled PA belong to other accession groups such as Trinitario and IMC
- Some accessions not labelled as PA fall in the PA group.

Table 3. Confirmed PA clones and identical duplicates at Marper Farm and UCRS.

Accession I	DNA sample number	Field plot location	Accession II	DNA sample number	Field plot location
PA 12 [PER]	FP424	Marper D 54	PA 12 [PER]	FP1106	6B D 200
PA 13 [PER]	FP821	Marper D158	PA 13 [PER]	FP823	Marper D159
PA 16 [PER]	FP189	Marper D573	PA 16 [PER]	FP189	6B D186
PA 24 [PER]	FP384	Marper D401	PA 24 [PER]	FP776	5B F507
PA 30 [PER]	FP1634	5B C144 T1	PA 30 [PER]	FP1632	5B C144 T5
PA 34 [PER]	FP333	Marper D259	PA 34 [PER]	FP653	5B E347
PA 45 [PER]	FP67	Marper D261	PA 45 [PER]	FP718	4A F528
PA 63 [PER]	FP1202	Marper D348	PA 63 [PER]	FP737	5A D265
PA 70 [PER]	FP278	Marper D634	PA 70 [PER]	FP791	5B F489
PA 84 [PER]	FP334	Marper D264	PA 84 [PER]	FP772	5B E388
PA 95 [PER]	FP280	Marper D663	PA 95 [PER]	FP658	5B F460
PA 105 [PER]	FP1179	Marper D285	PA 105 [PER]	FP762	4A F526
PA 113 [PER]	FP306	Marper D384	PA 113 [PER]	FP756	5A D307
PA 120 [PER]	FP194	Marper D318	PA 120 [PER]	FP627	6B D188
PA 121 [PER]	FP1185	Marper D393	PA 121 [PER]	FP636	6B C166
PA 124 [PER]	FP1251	Marper D492	PA 124 [PER]	FP629	6B D192
PA 126 [PER]	FP331	Marper D247	PA 126 [PER]	FP630	6B D198
PA 128 [PER]	FP388	Marper D357	PA 128 [PER]	FP739	5A D272
PA 137 [PER]	FP307	Marper D430	PA 137 [PER]	FP742	5A D274
PA 139 [PER]	FP308	Marper D426	PA 139 [PER]	FP722	4A F529
PA 141 [PER]	FP1249	Marper D463	PA 141 [PER]	FP28	5B F431
PA 149 [PER]	FP252	Marper D810	PA 149 [PER]	FP781	5B F474
PA 165 [PER]	FP239	Marper D714	PA 165 [PER]	FP785	5B F451
PA 175 [PER]	FP266	Marper D738	PA 175 [PER]	FP778	5B F473
PA 191 [PER]	FP265	Marper D743	PA 191 [PER]	FP777	5B F536
PA 196 [PER]	FP176	Marper D458	PA 196 [PER]	FP780	5B E371
PA 200 [PER]	FP111	Marper D710	PA 200 [PER]	FP792	5B F545
PA 279 [PER]	FP426	Marper D59	PA 279 [PER]	FP625	6B D197
PA 289 [PER]	FP559	Marper C803	PA 289 [PER]	FP793	5B F535
PA 291 [PER]	FP50	Marper D214	PA 291 [PER]	FP633	6B C167
PA 293 [PER]	FP258	Marper D762	PA 293 [PER]	FP566	Marper C817
PA 294 [PER]	FP1203	Marper D330	PA 294 [PER]	FP656	5B E389
PA 296 [PER]	FP162	Marper D495	PA 296 [PER]	FP631	6B D207
PA 299 [PER]	FP571	Marper C936	PA 299 [PER]	FP652	5B E398
PA 300 [PER]	FP382	Marper D544	PA 300 [PER]	FP771	5B E407
PA 303 [PER]	FP185	Marper D500	PA 303 [PER]	FP1084	6B D211

The Cluster analysis of the 158 DNA samples of PA labelled accessions using the DARwin software (Figure 2) provided additional information:

Figure 2. Dendrogram of dissimilarity analysis run on 158 PA accessions samples.



01 102

- 96 accessions labelled PA are grouped together in a cluster (Figure 2)
- Among the 41 pairs of original trees and their duplicates, 37 are matching (Table 3)
- Some PA labelled accessions and their homonymous duplicates, are not identical even though they belong to the PA group (Table 4a)
- Some PA labelled accessions and their duplicate trees were both found to be off-type (Table 4b)
- Some PA accessions share the same profile but bear different names (Table 4c)
- Some PA accessions have a duplicate which is an off-type (Table 4d)
- There is a cluster of “off-type” PA clones (Table 5).

Table 4a. PA confirmed accessions and PA non-identical duplicates.

Confirmed PA homonymous mislabelled clones					
Accession I	DNA sample number	Field plot location	Accession II	DNA sample number	Field plot location
PA 18 [PER]	FP425	Marper D55	PA 18 [PER]	FP1108	6B C145
PA 46 [PER]	FP1166	Marper D235	PA 46 [PER]	FP632	6B C159 T9

Table 4b. Confirmed off-type PA accessions and identical duplicates.

Confirmed off-type PA identical clones sampled from different plot and field					
Accession I	DNA sample number	Field plot location	Accession II	DNA sample number	Field plot location
PA 37 [PER]	FP1163	Marper D244	PA 37 [PER]	FP743	5A D253 T5
PA 65 [PER]	FP290	Marper D507	PA 66 [PER]	FP579	Marper C880
PA 66 [PER]	FP579	Marper C880	PA 66 [PER]	FP782	5B E356 T2
PA 27 [PER]	FP288	Marper D676	PA 194 [PER]	FP9	5B F513 T1

Table 4c. Confirmed PA identical accessions.

Confirmed PA synonymous mislabelled clones					
Accession I	DNA sample number	Field plot location	Accession II	DNA sample number	Field plot location
PA 124 [PER]	FP1251	Marper D492	PA 195 [PER]	FP628	6B C165 T1
PA 88 [PER]	FP294	Marper D677	PA 27 [PER]	FP774	5B E423 T4

Table 4d. Confirmed PA accessions and off-type duplicate.

Homonymous mislabelling					
Accession I	DNA sample number	Field plot location	Accession II	DNA sample number	Field plot location
PA 187 [PER]	FP387	Marper D482	PA 187 [PER]	FP228	Marper D737
PA 188 [PER]	FP238	Marper D724	PA 188 [PER]	FP790	5B F494 T9

Table 5. Distribution of the PA off-type accessions.

Accessions clustered with Trinitario accessions					
PA 37 [PER]	FP1163	Marper D225	PA 205 [PER]	FP46	Marper D715
PA 188 [PER]	FP790	5B F494 T11	PA 35 [PER]	FP2420	6B D225 T3
PA 167 [PER]	FP236	Marper D736	PA 275 [PER]	FP560	Marper C764
PA 114 [PER]	FP1580	5B F514 T3	PA 68 [PER]	FP10	5B E369 T14
PA 81 [PER]	FP1551	4A F527 T2	PA 841 [PER]	FP759	4A F517 T4

Accession clustered with IMC accessions		
PA 5 [PER]	FP613	Marper C1101

Other PA off-type accessions					
PA 1 [PER]	FP820	Marper	PA 65 [PER]	FP290	Marper D507
PA 66 [PER]	FP579	Marper C880	PA 187 [PER]	FP387	Marper D482
PA 27 [PER]	FP288	Marper D676	PA 194 [PER]	FP9	5B F513 T1
PA 143 [PER]	FP1960	5A D342	PA 310 [PER]	FP124	Marper D732

Mislabelling analysisPA labelled accessions

Trees with PA labels that we found to be off-types are examined below to search for feasible explanations of the mislabelling.

Trees showing a PA profile

PA 195 [PER] planted in UCRS Field 6B, plot C165 is a duplicate of PA 124 [PER]. The original PA 195 [PER] now missing, was in position Marper D493, adjacent to the original PA 124 [PER] clone in position D492. It is almost certain that budwood was mistakenly taken from PA 124 [PER] rather than PA 195 [PER].

PA 27 [PER] planted in UCRS field, plot E423 was not propagated from the clone PA 27 [PER] in Marper D676, but instead from the contiguous tree PA 88 in D677.

Trees showing a Trinitario profile

PA 37 [PER] from Marper D244 and the duplicate tree sampled from the UCRS both showed a Trinitario profile, implying that propagation was done from rootstock.

Whereas the PA 275 [PER] tree, Marper C764 shows a Trinitario profile, the neighbouring tree in C765, MOQ 6/29, shows a PA profile; the tree labelled PA 275 [PER] is rootstock and the tree labelled MOQ 6/29 is probably PA 275 [PER]. MOQ 6/29 was not propagated when the ICG,T was established at UCRS.

PA 205 [PER], Marper D715 and the neighbouring tree PA 167 [PER], Marper D736 have both been detected earlier by morphological observation to be rootstock. While PA 167 [PER], Marper D736 was not duplicated when the ICG,T was established, PA 205 [PER], Marper D715 and PA 275 [PER], Marper C764 were; morphological observation and DNA analysis should be performed to assess the identity of the replicate trees.

Accessions PA 35 [PER], UCRS Field 6B, plot D225 T3, PA 68 [PER], UCRS Field 5B, plot E369 T14, PA 81 [PER], UCRS Field 4A, plot F527 T2, PA 114 [PER], UCRS Field 5B, plot F514 T3 must have been all propagated from rootstock; the mother trees are now missing.

Tree 11 of PA 188 [PER] in UCRS Field 5B, plot F494 shows a Trinitario profile, although the fingerprint of the mother tree in Marper D724 shows the PA profile: this demonstrates that budwood for this tree was taken from the rootstock instead from the grafted tree. There is a need to authenticate the 14 other trees of the UCRS plot.

PA 841 [PER] had been already detected as being an off-type by morphological criteria and has been renamed CRU 4A/1.

Trees showing an IMC accession profile

The results of DNA analysis shows that the tree PA 5 [PER], Marper C1101 belongs to the IMC group. However, analysis of the DNA from the tree immediately next to it, CL19/49 infers that it belongs to the PA group and could be PA 5. The fact that the profile of the tree IMC 22 position C1102 matches NA 8, position C1058, makes plausible the explanation of the following mislabelling:

The tree in C1100 is PA 5 [PER] and the tree in C1101 is IMC 22, the tree in C1102 being a duplicate of NA 8, C1058 (Figure 3).

The duplicate trees in UCRS need to be checked.

Figure 3. Excerpt of the Block C field map in Marper Farm (trees are shown by position number/clone name).

1099/IMC 9	1100/CL19/49	1101/PA 5	1102/IMC 22
1057/B 8/9			
	1058/NA 8	SM 11	1059/B 16/1

Other PA off-type accessions

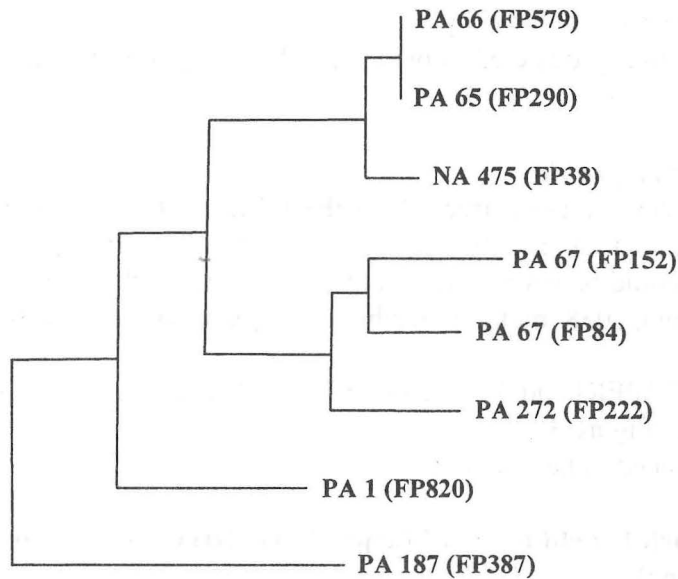
PA 3/10 [PER] (FP124) in Marper D732 is confirmed as an off-type; morphological observations had already led to the renaming of the tree as CRU 88. However the sample from PA 310 [PER] in UCRS Field 5A, plot D288, matches the PA group.

PA 143 [PER] (FP1960) collected from UCRS Field 5A, plot D342 T2 shows a profile that falls in the Refractario group. It is worth noting that the mother tree in Marper C985, now dead, was the first tree in the row, as was the case for PA 3/10 [PER]; there could have been some imprecision near the limits of the fields. A similar explanation could be given for the tree labelled PA 272 [PER], also an off-type, position D37 planted in the first row of the field.

In the dendrogram constructed with the DARwin software including all the 1,200 samples, 6 other off-type accessions were grouped in the same cluster as PA 272 [PER] (Figure 4). These clones are clearly separated from the PA group and there is some ambiguity about their sources for propagation in the ICG,T:

- PA 65 [PER] Marper D507 and PA 66 [PER] Marper C880 are duplicate trees.
- PA 194 [PER] planted in UCRS Field 5B was propagated from PA 27 [PER], Marper D676, which is an off-type. The accession labelled PA 27 [PER] in UCRS was collected from the neighbouring Marper tree PA 88 [PER]. PA 27 [PER], PA 88 [PER] and PA 194 [PER] were planted contiguously in Marper, but PA 194 [PER] has since died.

Figure 4. Excerpt of the dendrogram of dissimilarity analysis run on 1,200 DNA samples from cacao accessions in the ICG,T.



- Analysis of the data shows that PA 187 [PER], Marper D737 differs from PA 187 [PER], Marper D762. A question mark had been inserted in the notes for the tree in D737, dating from 1943.
- PA 272 [PER] and PA 1 [PER] are also off-types and have been propagated in UCRS: an assessment of all the duplicate trees is required.

Accessions not labelled as PA

The dissimilarity analysis (Figure 4) and the principal component analysis (Figure 1) of the 1,200 DNA samples shows that some accessions not labelled as PA, are genetically related to the PA clones (Table 6). These are shown as open points within the Parinari group in Figure 1.

Table 1. Original or duplicated accessions with a PA profile at Marper Farm and UCRS.

Clones with PA-like profile					
NA 423	FP262	Marper D757	MO 81	FP764	Marper D192
NA 759	FP32	5B H711 T15	IMC 41	FP1069	6B F418 T1
NA 851	FP21	5B F475 T2	IMC 81	FP1635	6B F421 T2
NA 534	FP11	5B G630 T1	B9/10-33 [POU]	FP299	Marper D632
NA 387	FP745	5A D251 T2	B21/6 [POU]	FP1204	Marper D395
NA 312	FP795	5B G614 T2	CL 19/49	FP1603	Marper C1000
NA 372	FP216	Marper D417	CL 19/51	FP66	Marper D27
NA 176	FP1662	4A D389 T4	MOQ 6/29	FP2103	Marper C765
NA 686	FP750	6A B105 T5	SCA16	FP284	Marper D671
MO 4	FP36	5B B111 T3	SLA 16	FP2707	5B D242 T8
MO 9	FP253	Marper D835			

Original trees potentially mislabelled

The MO 9 (FP253) DNA sample collected from the tree in Marper D835 shows a profile close to the adjacent accession PA 149 [PER] located in Marper D810. MO 9 could be a seedling of PA 149 [PER] as well as the 4 other unidentified surrounding trees, renamed CRU 134, CRU 135, CRU 136 and CRU 137.

The B 9/10-33 [POU] clone in Marper D632 shows a PA profile, and could be a seedling of the contiguous tree, PA 72 [PER] in Marper D633 that has a similar profile. This hypothesis of mislabelling is reinforced by the fact the tree in the next row (Marper D601), IMC 16 is also an off-type and shows a Nanay profile. The tree in Marper D602, now dead, was NA 105.

The analysis of B 21/6 [POU] (Marper D395) shows that this tree is a duplicate of the PA 140 [PER] tree planted in D439, just opposite and in the next row.

NA 423 (Marper D757) was planted next to PA 159 [PER], now dead, which could be its true identity. Similarly CL 19/51 in Marper D27 was the neighbour of a dead PA tree, as is NA 372 in D417.

The tree in Marper D671 bearing both labels SCA 16 and SLA 16, and its replicate tree in UCRS labelled SLA 16, share a PA profile; PA 31 [PER] and PA 26 [PER], now dead, were formerly planted in the vicinity.

The trees labelled MO 81 still present on the Campus fields should be sampled to compare with the MO 81 accession planted in Marper showing a PA profile.

Replicated mislabelled accessions

The dissimilarity analysis of DNA fingerprints shows that the MO 4 and IMC 41 accessions planted in UCRS are identical duplicates of a PA accession. Mother-trees of these accessions were planted contiguously in Marper D683 and D684, but are now dead. These trees could have been propagated from a seedling issued from one of the PA accessions nearby, PA 200 [PER] or PA 207 [PER]. Two trees CRU 86 and Marper 42, whose identities need to be clarified, are still alive in the vicinity.

The accession IMC 81 planted in UCRS field 6B, shares the same PA profile as the above, even though there is no evidence that such an accession was ever planted in Marper Farm; its identity was probably mistaken for IMC 41.

Tree 5 of the UCRS Field 6A, plot B105 shows a PA profile, although the fingerprint of the mother tree in Marper C383 shows the correct profile of NA 686. There is a need to authenticate the other NA 686 trees planted in fields 5A and 6A at UCRS.

The accessions NA 534 (FP11) and NA 387 (FP745) established in UCRS, share the same PA profile. The mother-trees, now dead, of these accessions were planted contiguously in Marper D781 and D782; the only living tree in the vicinity is an unidentified Marper 35 accession.

As NA 475 growing in Marper D469 was identified as being rootstock when collection of samples was undertaken, only the DNA from the NA 475 accession growing in UCRS 5B plot F534 was analysed. The result shows that budwood for propagation was taken from PA 65 [PER] located in the next row in Marper.

NA 176 growing in UCRS Field 4A plot D389 has the same profile as the original PA 176 [PER] accession planted in Marper D704. Mislabelling must have occurred at the time of the replication.

NA 312 growing in UCRS Field 5B plot G614 has the same profile as the PA 312 [PER] accession planted in UCRS Field 6B plot D209, although there is no record of an original tree in

Marper with this clone name.

Discussion and conclusion

From the genetic diversity revealed by the analysis of SSR profiles, Parinari accessions can be clearly identified as a distinct group of accessions.

Phenotypic diversity observed and analysed in CRU (Bekele *et al.*, 2005), has been reinforced by this work: for example, IMC 41 described as phenotypically closely linked to PA 171 [PER] and PA 303 [PER], has now been recognized as a member of the PA group by molecular analysis. “Low branching habit and dense canopy with long, dark leaves”, is shared by the PA 13 [PER] and PA 107 [PER] accessions (Bartley, 2005); the dissimilarity analysis of the DNA shows that these 2 clones are very closely linked.

The use of 15 markers has been efficient in completing the unambiguous identification of accessions amongst the group, and to detect population admixture. The analysis confirmed 96 PA clones as being correct, whilst only 6 cannot be included in the group.

For mislabelled accessions, feasible explanations can be found in most cases.

More verification of duplicated trees will be needed to reduce the risk of erroneous distribution from UCRS. It is valuable to know that the clones MO 4 and MO 9, selected to be propagated and later distributed to 13 producing countries as part of the CFC Project Collection, happen to be seedlings of PA clones.

Acknowledgements

We thank Antoinette Sankar for DNA sample preparation, Frances Bekele for sharing her knowledge, and the USDA-ARS Beltsville team for the efforts made to run the samples and to collect the data.

References

- Bekele, F.L. (2005) Examining phenotypic relationships among Upper Amazon Forastero clones. Pages 27-42, in: *Annual Report 2004*. St Augustine, Trinidad and Tobago: Cocoa Research Unit, the University of the West Indies.
- Bartley, B.G.D. (2005) *The genetic diversity of cocoa and its utilization*. Wallingford, UK: CABI publishing.
- Pound, F.J. (1943) First report on the selection of cacao trees for resistance to Witches' Broom disease. Unpublished report, Ministry of Agriculture, Trinidad.
- Saunders, J.A. (2000) USDA DNA Fingerprinting programme for identification of *Theobroma Cacao* accessions. Pages 108-114 in: *Proceedings of the international workshop on new technologies and cocoa breeding*, 16th-17th October 2000, Kota Kinabalu, Sabah, Malaysia. London, UK: INGENIC.
- Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N. and Bonhomme, F. (1996-2004) *GENETIX, logiciel sous Windows TM pour la génétique des populations*. Montpellier, France: Laboratoire Génome, Populations, Interactions CNRS UMR 5000, Université de Montpellier II.
- Perrier, X., Flori, A., Bonnot, F. (2003) Data analysis methods. Pages 43-76 in: *Genetic diversity of cultivated tropical plants*, (P. Hamon, M. Seguin, X. Perrier, J.C. Glaszmann Eds). Montpellier, France: Enfield, Science Publishers.