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Progress in resolving identity issues among the Nanay accessions held in Trinidad: the contribution of the collaborative USDA/CRU DNA Fingerprinting Project

M. Boccara and D. Zhang

During his expeditions to collect material with resistance to Witches' Broom disease, Dr. F.J. Pound collected pods from trees showing desirable traits along the Rio Nanay valley in Peru (Pound, 1943a, b). It is reported that pods were taken from 14 to 17 different trees, free of WB. After a suitable quarantine period in Barbados, healthy budwood from seedlings was grafted for establishment mostly in Marper Farm fields in Manzanilla. According to the listings of 1943, 340 trees from the Nanay group were planted in these fields, now called Blocks C and D. Sixty years later, 156 trees with Nanay labels are still alive in Marper Farm, and a total of 226 accessions have been duplicated in ICG,T and are recorded in the ICG,T database at CRU.

Mislabelling is a recurrent problem in every collection of living material and rigorous visual observations made over the years have suggested that some propagation errors were made (Bekele *et al.*, 2005), justifying the use of modern tools to resolve identity issues.

A joint USDA/CRU collaborative project that aims to obtain DNA fingerprints of cocoa germplasm held in the ICG,T started in 2001; the Nanay accessions being of special interest to the cocoa community, particular attention has been focused on that group.

Achievements

Leaves were collected from every original live tree in Blocks C and D of Marper Farm and from trees in UCRS where not present in Marper Farm. Extra leaf samples were also collected from replicated trees in UCRS for conformity control tests.

A total of 300 samples were collected, including 156 original trees in Marper Farm, 70 trees present only in UCRS fields and 74 for the purpose of verification. (Table 1)

DNA samples extracted in CRU were sent to the USDA-ARS Beltsville laboratory to be processed according to the planned protocol and guidelines (Saunders, 2000).

Data analysis

The results of the DNA fingerprinting profiles are currently available for a total of 1,300 accessions from UCRS and Marper Farm fields, including the NA accessions referred to in this study and have been analysed for different purposes:

- To assess the population identity of the Nanay group
- To assess the diversity of the group
- To detect off-type individuals
- To verify the conformity of duplicate trees
- To assess population admixture
- To discover potential mislabelling

Table 1. List of NA accessions sampled and their location in 2008.

Clone name	Marper Farm	Status	UCRS	Status	Clone name	Marper Farm	Status	UCRS	Status
NA 1	C1042	+	4A	+	NA 157	D649	0	--	
NA 3	C1045	+	--		NA 159	D650	+	--	
NA 8	C1058	+	--		NA 168	D435	+	5B	+
NA 12	C1048	+	--		NA 170	D478	+	--	
NA 13	C1091	+	6B	+	NA 176	--		4A, 5B	+
NA 14	C1047	+	--		NA 178	D434	0	--	
NA 16	C1093	+	5B	+	NA 179	--		5B	+
NA 19	--		4A	+	NA 181	--		6A	+
NA 21	D65	+	5A	+	NA 183	--		5B	+
NA 26	D584	+	5B	+	NA 184	D823	+	5B	+
NA 30	D97	+	--		NA 186	--		5B	+
NA 32	--		6B	+	NA 187	D816	+	6B	+
NA 33	--		4A	+	NA 189	--		5A	+
NA 34	--		6B	+	NA 191	--		5B	+
NA 39	D138	+	--		NA 194	D191	+	--	
NA 40	D120	+	--		NA 204	D476	0	--	
NA 43	--		5A	+	NA 206	--		6A	+
NA 45	D673	+	--		NA 217	D386	+	--	
NA 46	D157	+	5B	+	NA 218	D418	+	--	
NA 47	--		4A, 5B	+	NA 223	--		Campus	0
NA 48	D141	+	--		NA 226	--		6B	+
NA 49	--		4A	+	NA 227	--		5A	+
NA 58	--		5B	+	NA 228	--		5B	+
NA 61	D267	+	4A	+	NA 229	D483	+	--	
NA 62	D590	+	4A, 6A	+	NA 230	--		6B	+
NA 66	--		5B	+	NA 232	--		5B	+
NA 68	D135	+	5B	+	NA 235	--		5B	+
NA 69	D607	+	--		NA 241	D440	+	4A	+
NA 70	D129	+	--		NA 244	--		5B	+
NA 71	D695	+	5A	+	NA 246	D459	+	--	
NA 74	D206	+	--		NA 251	D490	+	--	
NA 79	D612	+	--		NA 254	D372	+	--	
NA 81	D221	+	--		NA 256	D269	+	--	
NA 84	D134	+	--		NA 258	D444	0	--	
NA 87	--		5B	+	NA 260	D341	+	--	
NA 90	D577	+	5B	+	NA 266	--		5B	+
NA 92	D608	+	5B	+	NA 268	D310	+	--	
NA 95	D222	+	--	+	NA 271	D270	+	5B	
NA 98	--		6B	0	NA 277	D313	+	--	
NA 1/19	D187	+	--		NA 279	--		5A	+
NA 104	D227	+	5B	+	NA 280	D838	+	--	
NA 106	D252	+	--		NA 283	--		5B	+
NA 110	D579	+	--		NA 286	--		5B	+
NA 111	D248	+	--		NA 289	--		5B	+
NA 112	--		5B	+	NA 300	--		5B	+
NA 113	--		4A, 5B	+	NA 311	--		5B	+
NA 114	--		4A, 5B	+	NA 312	D294	+	5B	
NA 118	--		6B	+	NA 320	--		5B	+
NA 127	D229	+	--		NA 322	D291	+	--	
NA 129	--		5A	+	NA 326	D289	+	--	
NA 137	D622	+	6B	+	NA 327	--		5B	+

NA 140	D349	+	--		NA 329	--		5A	+
NA 141	--		5B	+	NA 331	D477	+	--	
NA 142	D682	+	6A	+	NA 335	--		5B	+
NA 144	D626	+	--		NA 337	D822	+	--	
NA 145	D642	+	--	+	NA 339	--		4A	+
NA 149	D278	+	5B	+	NA 342	--		6B	+
NA 154	D624	+	--		NA 359	--		5B	+
NA 155	D276	+	--		NA 370	D783	+	--	
NA 156	D457	+	--		NA 371	D788	+	5B	
NA 372	D417	+	--		NA 718	C183	+	--	
NA 387	--		5A	+	NA 719	C228	+	5A	+
NA 395	D697	+	--		NA 720	C26	+	--	
NA 399	D456	+	--		NA 721	C234	+	--	
NA 406	--		5B	+	NA 724	C662	+	4A	+
NA 409	--		5A	+	NA 725	C675	+	--	
NA 423	D757	+	--		NA 726	--		Campus	+
NA 427	D466	+	--		NA 728	C434	0	--	
NA 432	D717	+	--		NA 730	D336	+	--	
NA 435	D760	+	5B	+	NA 732	C132	+	4A	+
NA 462	D784	+	5B	+	NA 733	D721	+	--	
NA 471	--		6A	+	NA 734	D546	+	--	
NA 475	D469	+	5B	+	NA 739	D193	+	5A	+
NA 504	D465	+	5A	+	NA 746	D213	+	--	
NA 507	--		5B	+	NA 747	D360	+	--	
NA 519	D808	+	5A	+	NA 750	--		6A	+
NA 528	D774	+	--		NA 753	C1160	+	--	
NA 534	--		5B	+	NA 756	D343	+	6A	+
NA 540	--		5B	+	NA 758	D219	+	6A	+
NA 669	C127	+	--		NA 759	--		5B	+
NA 670	--		5A	+	NA 763	D364	+	--	
NA 672	C133	+	5B	+	NA 764	D511	+	5B	+
NA 672	D538	+	5B	+	NA 766	D337	+	4A	+
NA 673	--		4A	+	NA 770	D496	+	5B	+
NA 674	C546	+	--		NA 771	--		5B	+
NA 675	C251	+	--		NA 773	--		5B	+
NA 678	C35	+	--		NA 780	D952	+	5B	+
NA 680	D716	+	5A	+	NA 794	D7	+	--	
NA 681	C663	+	--		NA 796	D272	+	5B	+
NA 685	C424	+	5B	+	NA 8/35	D368	+	--	
NA 686	C383	+	--		NA 802	D321	+	5A	+
NA 687	C78	+	--		NA 804	D320	+	6B	+
NA 689	C52	+	--		NA 807	D398	+	5A	+
NA 691	C415	+	--		NA 824	--		5B	+
NA 692	C693	+	--		NA 831	D741	+	--	
NA 693	C174	+	--		NA 833	D640	+	--	
NA 694	C64	+	--		NA 835	--		5B	+
NA 695	C47	+	--		NA 841	D698	0	--	
NA 697	C692	+	--		NA 847	D516	+	--	
NA 699	--		5B	+	NA 851	--		5B	+
NA 7/10	C181	+	6A	+	NA 860	D240	+	--	
NA 7/11	--		Campus	+	NA 867	D502	+	--	
NA 7/28	--		4A	+	NA 876	D486	+	--	
NA 702	D104	+	--		NA 877	D512	0	--	
NA 705	C102	+	5B	+	NA 888	D635	+	--	
NA 706	--		5B	+	NA 904	D523	+	--	
NA 708	C169	+	--		NA 91/6	D525	+	--	

NA 711	C659	+	--		NA 916	--		6B	+
NA 712	C247	+	--		NA 929	D499	+		+
NA 713	C275	+	--		NA 935	--		6B	+
NA 715	C89	+	5A	+	NA 937	D513	0	--	
NA 717	C608	+	--		NA 961	D637	+	5B	+

+ Trees alive and sampled

-- No tree(s) sampled

0 No DNA results

Methods

The following methodologies were used to analyse the data from DNA profiles:

- Genetic diversity of the 226 Nanay clones was assessed in relation to the 1,300 clones sampled from the ICGT, using dissimilarity analysis (DARwin software, 5.0.142, Perrier *et al.*, 2006) and principal co-ordinate analysis (GENETIX software, Belkhir *et al.*, 2000);
- Duplicate trees were assessed by matching their multilocus profile to their reference tree;
- Mislabeled trees and off-types were sought from matching profiles and by using all the information available in records, publications and maps.

Results

The principal co-ordinate analysis using Genetix software shows that Nanay accessions are clearly distinct from the rest of the clones analysed, but are grouped in two distinctive subgroups (I and II) (Figure 1). The main sub group includes 119 accessions (Table 2), whereas the other one includes 13 accessions (Table 3).

Table 4 shows also that some accessions labelled as Nanay fall into other accession groups mainly from Trinitario, Upper Amazon Forastero (*viz.*, Parinari accession group) and Refractario classes, and a few could be grouped with the Morona, IMC or Scavina accession groups.

Table 4. Distribution of the NA off-type accessions.

Clone name	Fingerprint code	Location	Clone name	Fingerprint code	Location
Clustered with Trinitario accessions			Clustered with Trinitario accessions		
NA 81	fp 1176	Marper D221	NA 320	fp 19	Field 5B E405 T1
NA 114	fp 1263	Field 5B E364 T2	NA 339	fp 721	Field 4A D404 T2
NA 142	fp 117	Marper D682	NA 395	fp 232	Marper D697
NA 159	fp 115	Marper D650	NA 462	fp 256	Marper D784
NA 260	fp 204	Marper D341	NA 540	fp 1568	Field 5B H641 T13
NA 271	fp 192	Marper D270	NA 691	fp 94	Marper C415
NA 277	fp 1191	Marper D313	NA 764	fp 386	Marper D511
NA 300	fp 12	Field 5B G601 T4	NA 961	fp 1010	Marper D637
Clustered with PA accessions			Clustered with PA accessions		
NA 176	fp 1662	Field 5B E403 T1	NA 534	fp 11	Field 5B G630 T1
NA 312	fp 663	Marper D294	NA 686	fp 750	Marper C383
NA 372	fp 216	Marper D417	NA 759	fp 32	Field 5B H711 T15
NA 387	fp 745	Field 5A D251 T2	NA 876	fp 131	Marper D486
NA 423	fp 262	Marper D757			
Clustered with Refractario accessions			Clustered with Refractario accessions		
NA 140	fp 313	Marper D349	NA 713	fp 104	Marper C275

NA 371	fp 241	Marper D788
NA 475	fp 38	Marper D469
NA 669	fp 102	Marper C127
NA 706	fp 39	Field 5B H692 T5
Clustered with Morona accessions		
NA 471	fp 1394	Field 6A B86 T9
NA 904	fp 313	Marper D523
Clustered with Scavina accessions		
NA 68	fp 1121	Marper D135
NA 145	fp 285	Marper D642
Clustered with IMC accessions		
NA 137	fp 1121	Marper D622

NA 732	fp 1310	Marper C132
NA 780	fp 565	Marper D952
NA 794	fp 397	Marper D7
NA 8/35	fp 199	Marper D368
Clustered with Morona accessions		
NA 91/6	fp 2717	Marper D525
Clustered with Scavina accessions		
NA 409	fp 104	Field 5A N4/526 T3
Clustered with IMC accessions		
NA 758	fp 104	Marper D219

Table 2. Confirmed NA accessions group I.

Clone name	Marper Farm	UCRS	Clone name	Marper Farm	UCRS
NA 1	C1042	4A	NA 329	--	5A
NA 8	C1058	--	NA 331	D477	--
NA 13	C1091	6B	NA 335	--	5B
NA 14	C1047	--	NA 337	D822	--
NA 16	C1093	5B	NA 342	--	6B
NA 19	--	4A	NA 359	--	5B
NA 26	--	5B	NA 370	D783	--
NA 30	D97	--	NA 395	D697	--
NA 32	--	6B	NA 399	D456	--
NA 33	--	4A	NA 406	--	5B
NA 34	--	6B	NA 427	D466	--
NA 40	D120	--	NA 432	D717	--
NA 43	--	5A	NA 435	D760	5B
NA 45	D673	--	NA 504	D465	5A
NA 46	D157	5B	NA 507	--	5B
NA 48	D141	--	NA 519	D808	5A
NA 62	D590	4A, 6A	NA 528	D774	--
NA 71	D695		NA 670	--	5A
NA 74	D206	--	NA 672	D538	5B
NA 84	D134	--	NA 674	C546	--
NA 90	D577	5B	NA 675	C251	--
NA 92	D608	5B	NA 678	C35	--
NA 95	D222	--	NA 680	D716	5A
NA 106	D252	--	NA 685	C424	5B
NA 110	D579	--	NA 687	C78	--
NA 111	D248	--	NA 689	C52	--
NA 112	--	5B	NA 697	C692	--
NA 127	D229	--	NA 699	--	5B
NA 141	--	5B	NA 7/10	C181	6A
NA 149	D278	5B	NA 7/28	--	4A
NA 154	D624	--	NA 702	D104	--
NA 155	D276	--	NA 705	C102	5B
NA 156	D457	--	NA 708	C169	--
NA 168	D435	5B	NA 715	C89	5A
NA 179	--	5B	NA 717	C608	--
NA 183	--	5B	NA 718	C183	--

NA 184	D823	5B	NA 719	C228	5A
NA 186	--	5B	NA 720	C26	--
NA 187	D816	6B	NA 724	C662	4A
NA 189	--	5A	NA 725	C675	--
NA 191	--	5B	NA 730	D336	--
NA 194	D191	--	NA 733	D721	--
NA 217	D386	--	NA 734	D546	--
NA 226	--	6B	NA 746	D213	--
NA 227	--	5A	NA 756	D343	6A
NA 228	--	5B	NA 766	D337	4A
NA 232	--	5B	NA 770	D496	5B
NA 235	--	5B	NA 771	--	5B
NA 244	--	5B	NA 773	--	5B
NA 246	D459	--	NA 796	D272	5B
NA 254	D372	--	NA 807	D398	5A
NA 266	--	5B	NA 824	--	5B
NA 279	--	5A	NA 831	D741	--
NA 280	D838	--	NA 833	D640	--
NA 283	--	5B	NA 841	D698	--
NA 289	--	5B	NA 847	D516	--
NA 311	--	5B	NA 867	D502	--
NA 322	D291	--	NA 888	D635	--
NA 326	D289	--	NA 929	D499	--
NA 327	--	5B			

Table 3. Confirmed NA accessions group II

Clone name	Marper Farm	UCRS	Clone name	Marper Farm	UCRS
NA 47	--	4A	NA 695	C47	--
NA 69	D607	--	NA 711	C659	--
NA 251	D490	--	NA 712	C247	--
NA 673	--	4A	NA 721	C234	--
NA 692	C1093	5B	NA 739	D193	5A
NA 693	C174	--	NA 747	D360	--
NA 694	C64	--			

Mislabelling analysis

Nanay trees presenting a Trinitario profile

The DNA profiles of 11 trees with Nanay labels in Marper Farm showed that they belong to the Trinitario group, implying that the surviving part of the tree is rootstock.

Some accessions in UCRS also presented a Trinitario profile: this is the case of NA 114 in Field 5B, which is identical to NA 114 in Marper Farm, implying that it was propagated from the surviving rootstock.

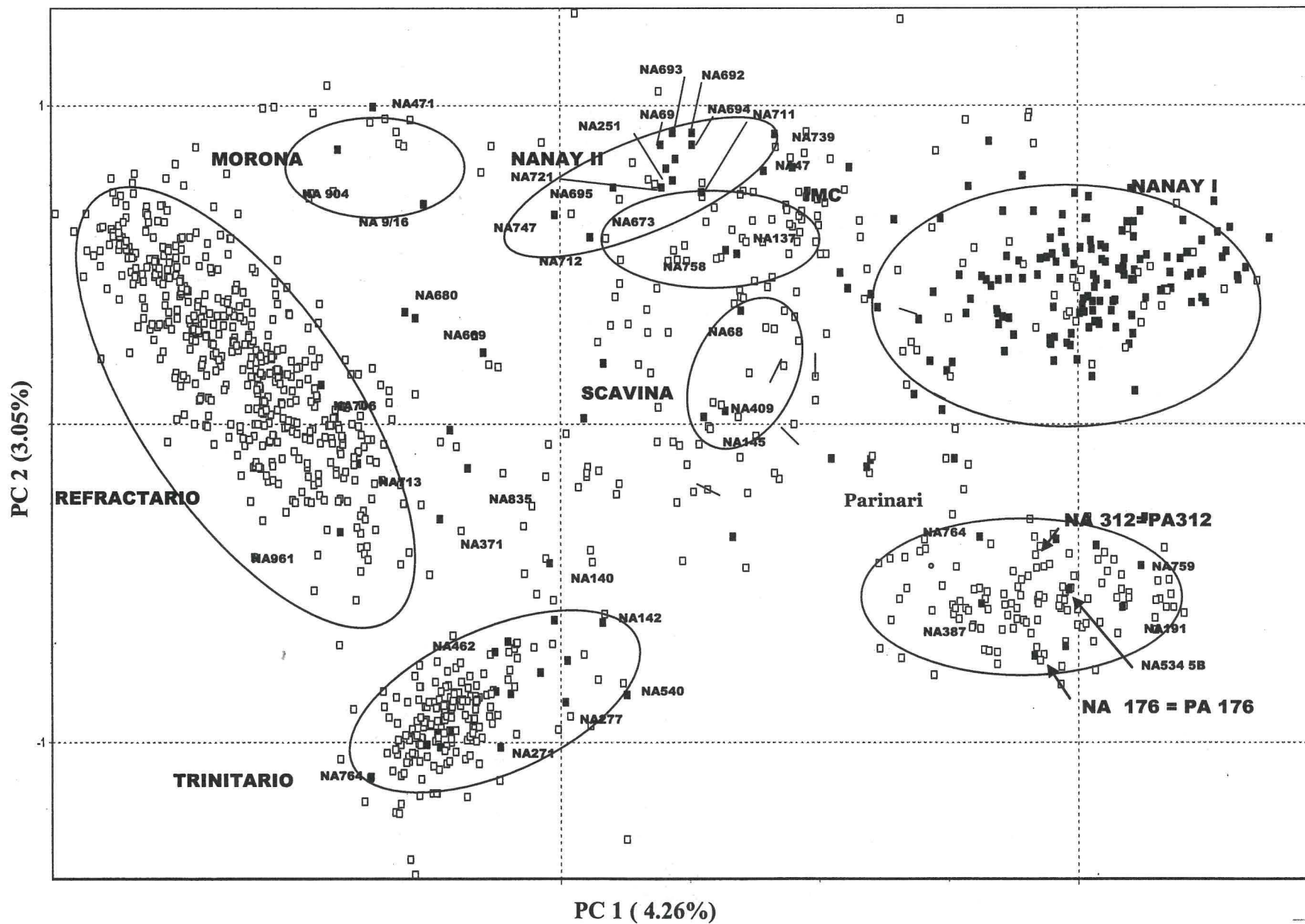
Trees presenting a PA profile

The results of the DNA analysis show that some trees labelled as Nanay in Marper Farm present the profile of another accession group (other than the Trinitario group); this is the case for NA 423 (Marper D757) which was originally planted next to PA 159 [PER] (Marper D756). The PA accession is now dead; this could be the true identity of the tree labelled NA 423.

Some duplicate trees in UCRS were propagated from the wrong original tree; this is often the

Figure 1. Principal component analysis for 1400 accessions from the ICG,T. Trees of Nanay group are shown as solid points.

43



case when the original tree was already dead at the time of establishment of the ICG,T; budwood was taken from an adjacent tree. This is the case for NA 387 in Field 5B probably propagated from the adjacent tree PA 111 [PER] (now dead).

Some mislabelling occurred during the propagation of trees in the establishment of the ICG,T: the plot D389 in Field 4A is labelled NA 176, but is planted with PA 176 [PER]; similarly the plot G614 in Field 5B is labelled NA 312, but contains PA 312 [PER] trees.

Trees presenting a IMC profile

NA 758 collected in Marper D219 shows the profile of IMC 38 established in Marper D681; although another IMC 38 was planted in D218, only the rootstock was reported to be still alive: the DNA analysis shows that the tree labelled NA 758 is fact IMC 38.

Trees presenting a Scavina profile

NA145 collected in Marper D642 shows a Scavina profile. As it had been planted next to SCA 8, now dead, in Marper D643 but does not match exactly the profile of the SCA 8 alive on the UWI campus, the tree labelled NA 145 could be a seedling from SCA 8 now dead in Marper.

Trees presenting a Refractario profile

The analysis of the profiles of the group of accessions clustered with Refractarios (Table 4) shows that they cluster with all the accessions labelled SM [POU]. Assumptions were made about the origin of this group of trees, (collected from a farm San Miguel across Rio Vines (Bartley, 1993, cited in ICGD, Wadsworth *et al.*, 1997), or from Los Rios). A possible explanation could be that the group of NA accessions, together with SM [POU] accessions were collected along the lower part of the Nanay river from a place called San Miguel, close to Iquitos (3°50'S 73°15'W).

Discussion and conclusion

From the genetic diversity revealed by the analysis of DNA profiles, NA accessions can be identified as a distinct origin. However, it appears that 2 sub-groups can be discerned: this finding could corroborate the hypothesis that some of the accessions (Group II) could be the "result of the generation of seedling progenies of the original collections" given in the CRU Annual Report for 2004 (Bekele *et al.*, 2005).

The use of the 15 SSR markers has been efficient in distinguishing most of the NA accessions in the group. However, few very homozygous accessions cannot be clearly differentiated, even with the use of additional markers.

As it has been shown by Motilal (2008) that plots in the ICG,T can contain more than one tree group, more verification of duplicate trees in the ICG,T will be needed to confirm their identity.

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