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Germplasm Enhancement
for Black Pod resistance

established-2001

FIELD 7

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

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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@sta.uw.edu

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Identity assessment of Refractario origin cocoa accessions held in Trinidad: the contribution of the collaborative USDA/CRU project

M. Boccara and D. Zhang

Introduction

“Refractario cacao” originated from a large group of germplasm collected during the 1920s from the coastal region of Ecuador and selected for its potential resistance to Witches’ Broom disease. Seedlings from fruits of these trees were raised in nurseries, screened for resistance to the disease and then established in various farms in Ecuador. Dr F. J. Pound collected pods from 9 farms selected from “some” 80 trees free of Witches’ Broom disease symptoms, and seeds were planted in Barbados for quarantine purposes (Table 1). After a suitable period, healthy budwood from the seedlings was forwarded to Trinidad, budded onto rootstock and subsequently planted mostly in Marper Farm and a few on the ICTA Campus.

Records available in CRU show that more than 1,250 Refractario clones were present at one time in Marper Farm: 1,000 in Block C and 250 in Block D. Other Refractario trees were also planted on the ICTA campus and among them 6 clones that were not represented in Marper.

When the ICG,T was established from 1986 to 1994, 746 plots of Refractario origin trees were planted in UCRS.

Currently, in Marper Farm, 716 Refractario trees are still alive, 599 in block C, 117 in Block D and in UCRS, 544 clones replicated in 746 plots.

An international collaborative project on DNA fingerprinting of cocoa germplasm was started in 2001, and priority was given to the analysis of material collected by Pound such as the “Refractarios” since they are of special interest to the international cocoa community.

Table 1. Refractario accessions inventory and their origin.

Farm	Group name	Trees planted		Remaining trees		
		Marper C	Marper D	Marper C	Marper D	UCRS only
Amalia	AM	116	12	79	11	4
Balao	B	67	96	37	41	11
Clementina	CL, CLM	179	32	116	11	19
Javilla	JA	203	30	130	14	12
Large Vuelta	LV, LX, LZ	27	13	13	9	2
La Paz	LP	140	15	83	8	14
Moquique	MOQ	188	24	86	10	15
Santa Lucia	SLA, SLC	37	22	23	11	5
San Juan	SJ	47	5	32	2	6
Total		1004	249	599	117	88

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 extra leaf samples was also undertaken for verification purposes.

A total of 802 samples were collected, including 716 from original trees from Marper fields, 67 from UCRS trees now absent from Marper as well as 41 samples from replicated trees in UCRS to check their conformity.

DNA was extracted in CRU and samples were sent to the USDA Beltsville laboratory for analysis with 15 selected SSR primers, following the recommended protocol and guide-lines (Saunders, 2000).

Data analysis

The results of the DNA profiles from USDA-ARS¹ Beltsville laboratory are available for 1,200 clones from CRU, including 590 Refractario clones and have been used for different purposes:

- To assess the population identity of the Refractario group
- To detect off-type clones of the population
- To verify that duplicate trees are identical
- To place individual trees in appropriate half-sib families
- To assess population admixture
- To discover potential mislabelling and to find conceivable explanations

Methods

Genetic diversity of the 590 Refractario 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 (GENETIX software, v.4.03).

Duplicate trees were assessed by identifying matching multilocus genotypes among Refractario 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

Genetic diversity of the Refractario clones and potential mislabelling

The principle component analysis (PCA) (Figure 1) shows clearly that the Refractario accessions form a group that is distinct from the rest of the clones analysed. It also shows that some accessions labelled Refractario belong to other accession groups mainly Trinitario (Table 2), but

¹ USDA – Agriculture Research Service

Figure 1. Principal component analysis for 1,200 accessions from the ICG,T. Trees of Refractario origins are shown as solid points.

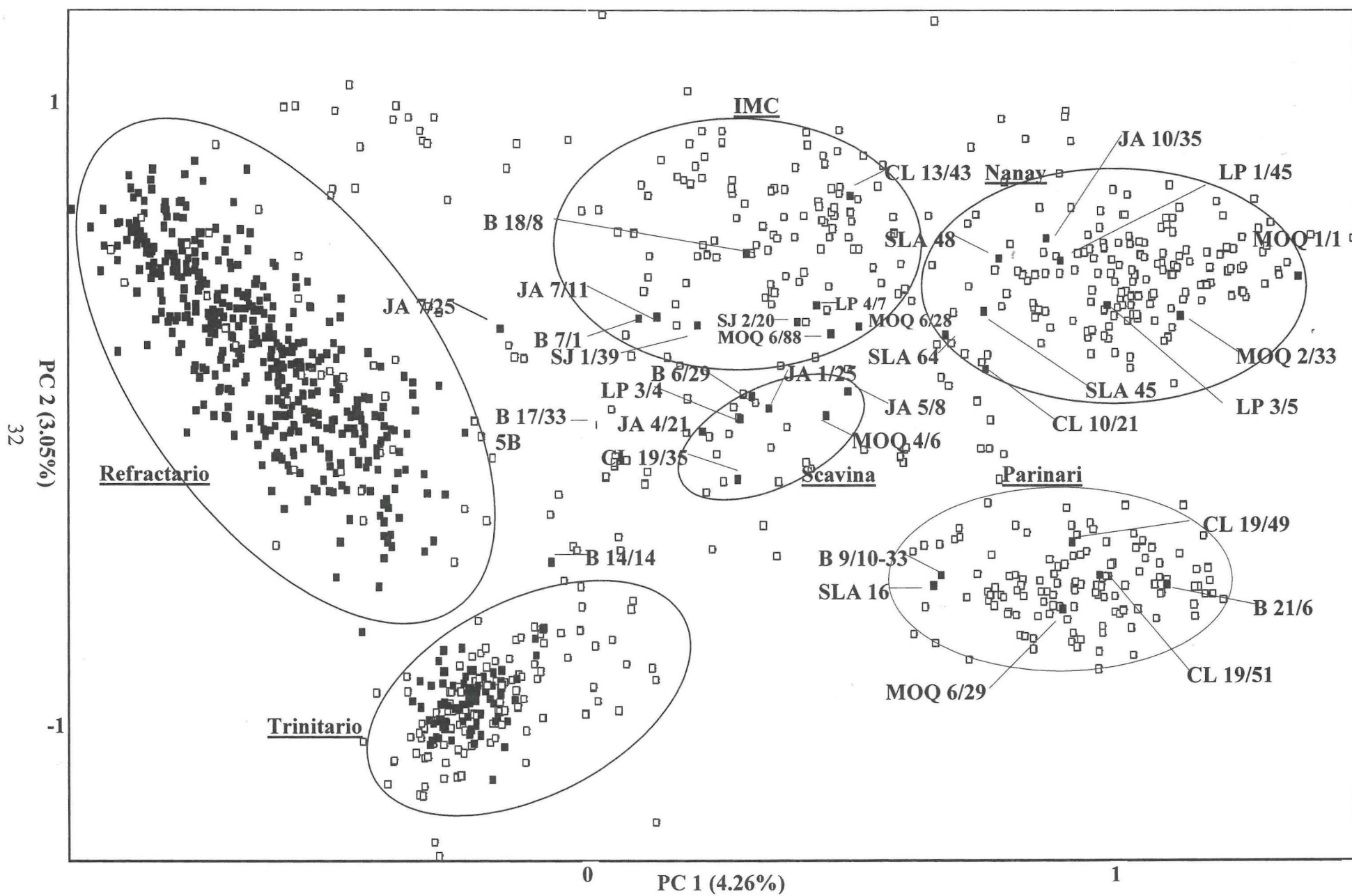


Table 2. List of off-type Refractario accessions that group with Trinitario genotypes.

Accessions clustered with Trinitario accessions					
Clone name	Fingerprint code	Location	Clone name	Fingerprint code	Location
AM 1/28 [POU]	fp702	Marper C189	AM 2/61 [POU]	fp1336	Marper C 278
AM 1/73 [POU]	fp2147	Marper C925	AM 2/64 [POU]	fp1565	Marper D739
AM 1/96 [POU]	fp412	Marper D28	AM 2/90 [POU]	fp264	Marper C911
B 1/2-24 [POU]	fp1126	Marper D155	B 13/6 [POU]	fp73	Marper D42
B 21/7 [POU]	fp371	Marper D580	CL 10/14 [POU]	fp 2005	Marper C370
CL 10/27 [POU]	fp214	Marper D296	CL 13/17 [POU]	fp569	Marper C67
CL 13/36 [POU]	fp2148	Marper C930	CL 13/4 [POU]	fp1321	Marper C299
CL 19/2 [POU]	fp603	Marper C36	CL 19/21 [POU]	fp2244	Marper C707
CL 19/22 [POU]	fp1360	Marper C279	CL 19/41 [POU]	fp2039	Marper C519
CL 27/21 [POU]	fp2024	Marper C461	CL 27/58 [POU]	fp1322	Marper C224
CL 9/13 [POU]	fp1305	Marper C43	CL 9/16 [POU]	fp2241	Marper C666
CL 78/3 [POU]	fp1362	Marper C277	CLM 3 [POU]	fp2015	Marper C394
CLM 43 [POU]	fp2025	Marper C454	CLM 64 [POU]	fp2177	Marper C768
CLM 99 [POU]	fp2159	Marper C906	JA 1/14 [POU]	fp2437	Marper C1001
JA 1/8 [POU]	fp1304	Marper C42	JA 10/5 [POU]	fp2248	Marper C630
JA 3/20 [POU]	fp2444	Marper C699	JA 3/22 [POU]	fp1853	Campus 11
JA 3/30 [POU]	fp2084	Marper C1106	JA 4/9 [POU]	fp2441	Marper C717
JA 5/28 [POU]	fp1852	Campus 11	LP 1/24 [POU]	fp2252	Marper C724
LP 2/17 [POU]	fp2169	Marper C925	LP 3/15 [POU]	fp2467	Marper C307
LP 3/20 [POU]	fp2048	Marper C691	LP 3/48 [POU]	fp2393	Marper C821
LP 4/32 [POU]	fp2256	Marper C752	LP 6/16 [POU]	fp2055	Marper C538
LP 6/19[POU]	fp1193	Marper D317	LX 18 [POU]	fp685	Marper C138
LX 25 [POU]	fp814	Marper D105	LX 31 [POU]	fp353	Marper D113
LX 45 [POU]	fp68	Marper D78	MOQ 1/14 [POU]	fp249	Marper D793
MOQ 1/25 [POU]	fp1592	Marper C956	MOQ 4/17 [POU]	fp2174	Marper C984
MOQ 4/2 [POU]	fp697	Marper C144	MOQ 4/20 [POU]	fp136	Marper D799
MOQ 5/34[POU]	fp2198	Marper C795	MOQ 6/102[POU]	fp2635	Marper C846
MOQ 6/103[POU]	fp2156	Marper C931	MOQ 6/19 [POU]	fp1297	Marper C116
MOQ 6/36 [POU]	fp2335	Marper C462	MOQ 6/41[POU]	fp1332	Marper C182
MOQ 6/67[POU]	fp684	Marper C140	MOQ 6/77 [POU]	fp2315	Marper C756
MOQ 6/87 [POU]	fp558	Marper C4	MOQ 6/93 [POU]	fp2450	Marper C839
SJ 1/29 [POU]	fp2061	Marper C410	SJ 2/16 [POU]	fp577	Marper C5
SJ 2/17 [POU]	fp589	Marper C33	SJ 2/19 [POU]	fp1369	Marper C337
SJ 2/30 [POU]	fp2376	Marper C909	SLA 13 [POU]	fp1370	Marper C230
SLA 20 [POU]	fp1340	Marper C231	SLA 64 [POU]	fp2071	Marper C1163
SLC 3 [POU]	fp2066	Marper C508	SLC 18 [POU]	fp822	Marper D183

also Parinari, IMC, Nanay, or Scavina. (Table 3).

The Cluster analysis of the 590 DNA samples of the Refractario labelled accessions was performed using the DARwin software (Figure 2) and provided additional information:

- 480 accessions labelled Refractario are grouped together in a cluster
- Some Refractario accessions share the same profile but were labelled differently
- Some Refractario accessions have a duplicate which is an off-type

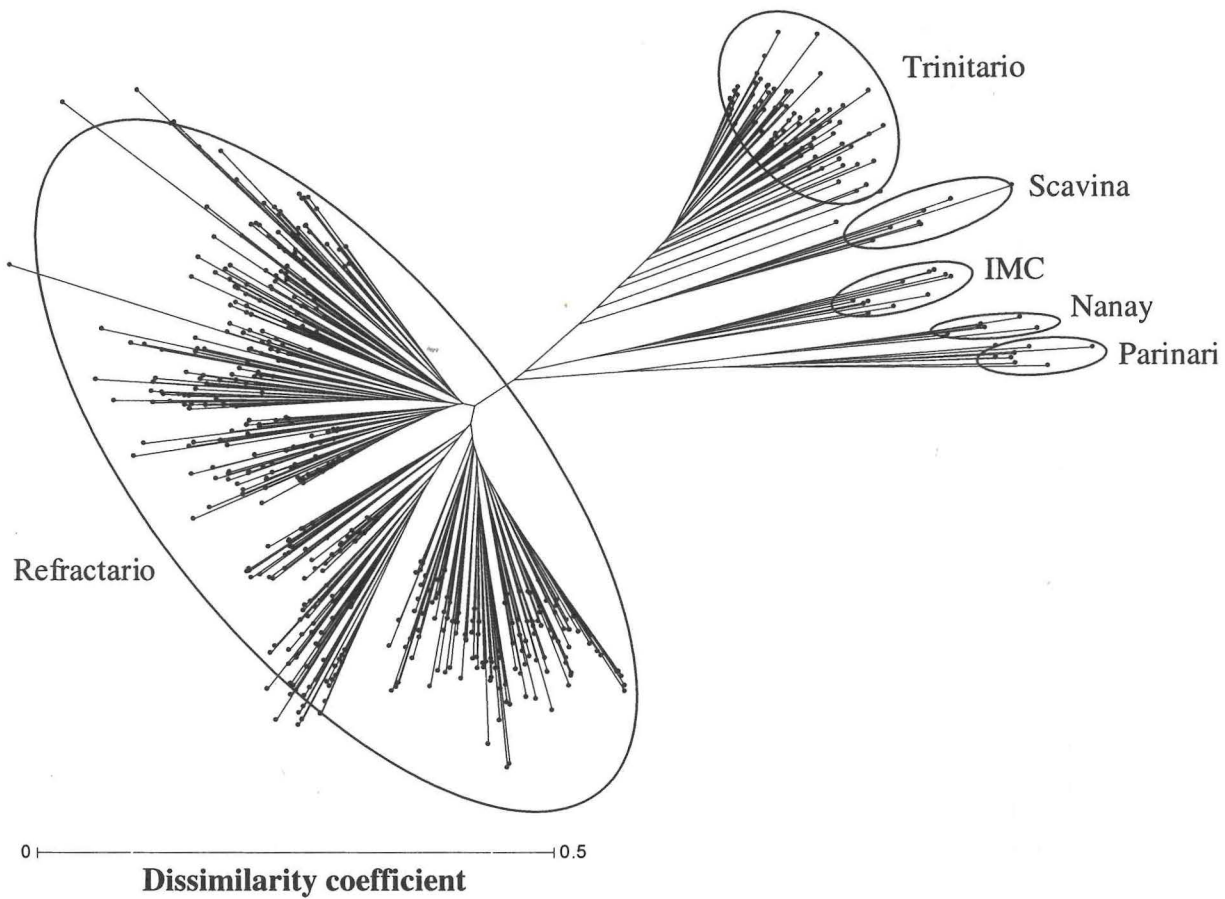


Figure 2. Cluster analysis of 590 DNA samples from trees with labels of the Refractario group.

Mislabelling analysis

Refractario trees presenting a Trinitario profile.

The DNA profiles of 72 accessions with Refractario labels show that they belong to the Trinitario group, implying that the tree or portion of it still alive in the field is constituted of rootstock.

Trees presenting a Refractario profile

The analysis of AM 1/19 [POU] (Marper C922) and AM 2/92 [POU] (Marper C923) profiles show that these trees are identical; this is also the case for AM 2/70 [POU] and LX 20, established just opposite and in the next row, inferring that labels were misplaced.

Table 3. List of identified off-type Refractario accessions.

Clone name	Fingerprint code	Location	Clone name	Fingerprint code	Location
Accessions clustered with IMC accessions					
B 7/1 [POU]	fp1382	5A B80 T15	B 17/33 [POU]	fp1378	5A B78 T8
B 18/8 [POU]	fp41	Marper D173	CL 13/43	fp1254	5B A63 T9
JA 7/11 [POU]	fp1710	5B F521 T2	LP 4/7 [POU]	fp2257	Marper C624
MOQ 6/28	fp2699	4A D357 T1	MOQ 6/88	fp1850	Campus 11
SJ 1/39 [POU]	fp2294	Marper C428	SJ 2/20 [POU]	fp1695	5B B131 T5
Accessions clustered with NA accessions					
CL 10/21	fp578	Marper C9	JA 10/35 [POU]	fp1596	Marper C1002
LP 1/45 [POU]	fp291	Marper D385	LP 3/5 [POU]	fp2357	5B B86 T6
MOQ 1/1	fp1390	6A B83 T13	MOQ 2/33	fp2193	Marper C781
SLA 45	fp1957	5A D299 T10	SLA 48	fp666	Marper D88
SLA 64	fp2695	Marper C1165			
Accessions clustered with PA accessions					
B 9/10-33 [POU]	fp299	Marper D632	B 21/6 [POU]	fp1204	Marper D395
CL 19/49	fp1603	Marper C1100	CL 19/51	fp66	Marper D27
MOQ 6/29	fp2103	Marper C765	SLA 16	fp2707	5B D242 T8
Accessions clustered with SCA accessions					
B 6/29[POU]	fp1381	5A B81 T14	CL 19/35	fp1361	Marper C328
JA 4/21 [POU]	fp416	Marper D2	JA 1/25 [POU]	fp1413	5B D287 T4
JA 5/8 [POU]	fp1874	Campus 11	LP 3/4 [POU]	fp2311	Marper C523
MOQ 4/6	fp1563	5B H680 T13			
Other Refractario off-type accessions					
B 14/14 [POU]	fp1572	5B A44 T11	JA 7/25 [POU]	fp1414	5B G567 T6

Trees presenting a non-Refractario profile

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, next row.

SLA 48 (Marper C86) shows a Nanay profile: it had been planted next to NA 22, believed to have died, which could be its true identity. Similar situations are found for B 18/8 [POU] (Marper D173) which could be either IMC 75, IMC 64 or IMC 38; LP 1/45 [POU] (Marper D385) which could be NA 163 or NA 332; CL 19/51 (Marper D27) which could be PA 281 [PER]; B 9/10-33[POU] (Marper D632) which could be PA 72 [PER], and LP 3/4 [POU] (Marper C523) which could be PA 35 [PER].

Some duplicate trees in UCRS were propagated from the wrong original tree. It happened usually in the case where the original tree was already dead when the ICG,T was established: budwood for cuttings was taken from an adjacent tree. The analysis shows that this occurred for accessions B 7/1 [POU], SJ 2/20 [POU] and LP 3/5 [POU].

B 6/29 [POU] tree 14, from Field 5A shows a Scavina profile whereas the DNA analysis of the original tree, still alive in Marper, matches a Refractario identity. Verification of the other trees duplicated in the same plot must be performed.

Mislabelling of plots in UCRS

Matching of some DNA profiles allows us to detect some incorrect labelling of UCRS plots. This is the case of plot B83 in Field 5A labelled B 10/28 [POU] instead of B 9/10-28 [POU], plot A45 in Field 5B labelled B 2/34 [POU] instead of B 23/4 [POU], plot B104 in Field 5A labelled CL 7/89 instead of CL 78/9, plot E473 in Field 4A labelled LP 4/45 [POU] instead of LP 4/41 [POU] (the original LP 4/45 [POU] tree in Marper Farm being dead), as well as plot D350 in Field 4A labelled MOQ 1/24 instead of MOQ 1/22.

Discussion and conclusion

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

The use of 15 markers has been efficient in completing the unambiguous identification of accessions amongst the group.

The analysis confirmed the membership of 480 clones to the Refractario group, and revealed that 72 trees in Marper Farm with Refractario labels were rootstock. It has been reassuring that not more than 40 other accessions were identified as off-types.

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

More verification of duplicate trees will be needed to reduce potential errors in material distributed from UCRS.

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