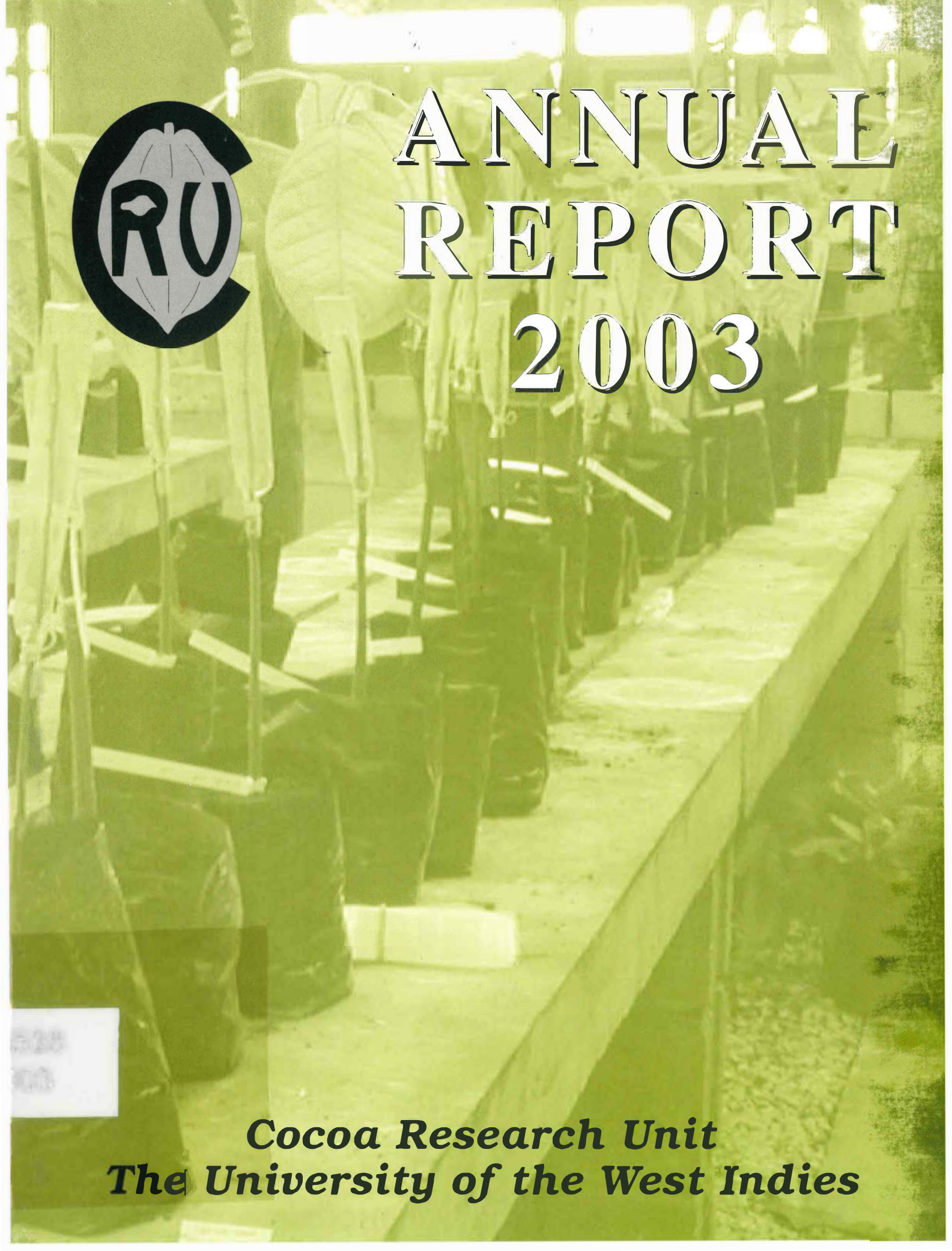

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Cover photograph. Multiplication of accessions in the Barbados Cocoa Quarantine Station.

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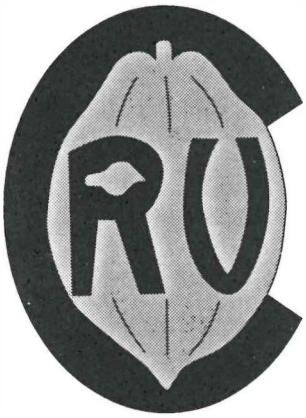

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Resolving identity issues of cocoa clones using SSR markers

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Introduction

The ICG,T is an internationally recognised field genebank for cacao and represents an important resource for the world cocoa community. It contains accessions from the early expeditions collected in the 1930s as well as more recent introductions and many local selections. The need for correct identification of every tree in a genebank is imperative (this is highlighted when, for example, information which is gathered from different sources shows discrepancies). Molecular characterisation of cocoa germplasm with the use of SSR markers is a dependable way to confirm identity and to correct identification errors.

Verification has always been an ongoing task in CRU as it is the base of origin for many cultivars grown worldwide.

Material and methods

For the majority of accessions in UCRS, the original tree representing an accession is either in Marper Farm, the Cheesman Field in the San Juan Estate or in the UWI Campus. The creation of the ICG,T at La Reunion Estate was done by planting rooted cuttings or grafted trees into plots containing 4 to 16 trees. Unfortunately, in some cases the identity of the original tree was lost, mislabelling occurred and/or errors were made during the planting process or the drawing of maps.

The USDA/CRU fingerprinting project gave us the opportunity to sample DNA from the most original tree of each accession held in Trinidad. These samples are used as a standard reference for duplicates of each clone.

A modified Kobayashi extraction method (Kobayashi *et al.*, 1998) was used as a standard routine to obtain the DNA. The template quantification was achieved in a Turner Biosystems mini-fluorometer with the Hoechst 33258 dye (Anon 2004).

The accessions were checked by running electrophoresis on 3% agarose gels, stained with ethidium-bromide and photographed under UV light. Eight SSR primers were used to complete the comparisons between samples.

Results and discussion

IMC 47

IMC 47 is a clone present in 20 research centres across the world, but some verification work has shown that clones differed in multiple profiles.

In Trinidad, IMC 47 trees are present in different locations:

- One tree in Marper Farm, position D 242
- One tree in UWI Campus 11, coordinates x5y12
- Eleven trees in Field 6B, plot F401 at UCRS

- Two trees in Field 6B (T10 and T14) had been verified and considered as true to type by CIRAD (Risterucci, 2001).

Our verification has included the tree from Marper Farm, the tree from UWI Campus and several trees from Field 6B at UCRS.

Results

Although the tree in Marper Farm is different from the one on Campus, trees 10, 13 and 14 in Field 6B have a similar profile to the Campus tree; trees 1, 3, 4, 11, 12 share the same profile, but are different to both the Marper and the Campus tree profiles. Preliminary results from Reading University indicate that RUQ 849 which is the IMC 47 held in the Reading quarantine facility has an identical profile to the Campus tree.

ICS 83 and ICS 95

These clones are included in the CFC/ICCO/IPGRI Project Collection. DNA samples from trees in the Cheesman Field (ICS 95 Block 2 and ICS 83 Block 5) were sent to USDA Beltsville for the fingerprinting project and have been analysed.

Results

The preliminary results provided by USDA have shown that ICS 83 and ICS 95 could be duplicates, as they are sharing the same profile. DNA analysis from a tree of the ICS 83 plot in UCRS has a different profile to both the San Juan Estate trees. This shows an example of mislabelling and/or planting error, as ICS 83 and ICS 95 are neighbours in block 5.
The pods of ICS 83 in UCRS are partially pigmented whereas the pods of ICS 95 are very dark red, matching Pound's descriptions (Pound, 1936). It follows that budwood for the propagation of ICS 83 should be taken from the UCRS plot.

ICS 45 and ICS 46

The only ICS 45 tree remaining in Cheesman Field, although standing at the correct position according to the map, bears an old label, which reads ICS 46. There is a reference ICS 46 tree in Block 2.

Results

The experiment has shown by comparison of profiles that this tree is not identical to the ICS 46 tree present in Block 2, and is likely to have been mislabelled.

MOQ 1/12 and CRU 10

Two trees in Marper Farm Block C (positions C205 and C259) are labelled as MOQ 1/12. According to hand written records from 1943, the tree in position C260 is also a MOQ 1/12; however, the most recent map and listing refer to this tree as CRU 10.

Results
SSR analysis has shown that the trees in C259 and C260 share the same profile; on the other hand, the C205 tree shows a different profile. According to this evidence, the C260 tree should be re-assigned the name MOQ 1/12, whereas the tree in C205 should be re-named.

MOQ 2/18 and MARPER 7

The tree originally planted in Marper C784 was MOQ 2/18, but the tree in this position was later named MARPER 7, since the original identity had been lost. There are two trees in UCRS Field 5B, Plot C171, labelled as MOQ 2/18, and one in Campus 3, coordinates x10y10.

Results
Preliminary results have shown that tree 10, from 5B, MOQ 2/18 from Campus, and MARPER 7, all show different profiles.

AM 2/88 [POU] and MARPER 10

According to the 1943 records, the clone AM 2/88 [POU] was established in two locations in Marper, C895 and D404; since then the tree in C895 has been renamed MARPER 10.

Results
Differences were found in SSR profiles (differ in 5 out of 8): the distinction between these two trees has been confirmed.

CL 9/11 and CL 91/1

The tree planted in Marper Farm, position C520, was recorded as CL 9.11, while the one in position C678 has been listed as CL 911. The trees propagated in UCRS 4A, named CL 9/11, could have originated from one location or the other.

Results
Although the tree in C678 is now missing, the fact that the profile of the trees in 4A match that from MARPER C520 for 8/8 SSR primers, suggests that the source tree was Marper C520.

SLA 16, SCA 16 and SLA 23

The tree planted in position D671 in Marper Farm, listed as SCA 16 in the 1943 records, bears a SLA 16 label (probably due to confusing handwriting in the early records). Plot D242 in Field 5B is labelled SLA 16, although an old map of 5B shows this plot as SLA 23.

Results
SSR analysis showed that DNA from the original tree in Marper D671 matches the tree sampled in Plot D242 Field 5B. The DNA of the SLA 23 reference tree from Marper is dissimilar, thus confirming that the tree in 5B is probably SCA 16.

CL 9/17 and CL 19/17

The 1943 records from Marper Farm list two trees in position C56, one is CL 9.17 and the other is CL 19.17. Although the updated map names the tree in this position as CL 9/17, the trunk bears a CL 19/17 label.

DNA samples from the Marper C56 tree, from a CL 9/17 tree in Field 5B plot A24, and from a CL 19/17 tree in Field 5B plot I731 were checked.

Results

SSR profiles from trees in the two plots in Field 5B (A24 and I731) are the same but both differ from the Marper C56 tree. It is possible that the trees in 5B were propagated from the adjacent CL 19/17 tree, now missing.

Conclusion

Analyses of SSR profiles have allowed us to resolve issues of identity ambiguity for a selection of trees and clones in the ICG,T fields. Improper naming or labelling can be corrected and updated.

This new information is of particular value for accessions that are already widely distributed, or for those which have been selected for current research activities, and may be distributed in the future.

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