

RELIABILITY OF CONSTANT TRAITS IN MORPHOLOGICAL

CHARACTERIZATION OF CASSAVA AS COMPARED TO AFLP MARKERS

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ABSTRACT A prerequisite for any genetic improvement programme is knowledge of the extent of genetic variation present between cultivars and genetic distances between them. This can be achieved through characterisation of germplasm either using morphological, biochemical or DNA markers. This study was done to compare morphological and DNA based molecular marker (AFLP) technologies in characterising cassava genotypes. Trials with 16 cassava genotypes were conducted from 2000 to 2002 at Chitedze and Makoka Research Stations in Malawi, while the DNA fingerprinting was done at the UFS. Genetic distances determined by morphological characterisation using mainly constant traits correlated to similar values using AFLP fingerprinting.

INTRODUCTION The starchy roots of cassava have become the most important source of dietary energy in Sub-Saharan Africa (FAO, 2000; Scott et al., 2000). This is due to its high and stable yield. Other advantages of cassava include its flexibility in planting and harvesting time. Cassava is grown in almost all areas, and it is the most important root crop in Malawi (Benesi et al., 1999). Small-scale farmers play a crucial role in maintaining a broad genetic variability of cassava (Brush, 1995). A prerequisite for genetic improvement of cassava, is a knowledge of the extent of genetic variation (Beeching et al., 1993). Hence, the objective of this study was to compare the use of morphological and molecular data in characterising cassava genotypes.

MATERIALS AND METHODS The recommended varieties (Mbundumali, Chitembwere, Maunjili, Silira, Sauti, Yizaso and Mkondezi), locally bred promising clones (CH92/105 and CH92/082), and introduced clones (TME1, 30786, TMS60121, 83350, LCN8010 and TMS60142A) were planted Chitedze and Makoka research stations 2000/01 season. The plot sizes were four ridges, each with 12 plants. The ridges were 0.9 m apart and the plants were also 0.9 m apart along the ridge. The AFLP fingerprinting was done at the UFS.



Figure 1: Morphological characterisation being done right in the field, normally carried out as a team



Figure 2a: Constant traits like petiolar colour, leaf shape, shoot tip colour, hairiness of unexpanded leaves, leaf colour, etc



Figure 2b: Constant traits like stem colour and root colour, as well as those in Figure 1a are crucial in characterisation

Morphological characterisation was done using the modified IBPGR descriptors (Nweke et al., 1994; Mahungu and Kanju, 1997). Constant traits were emphasised. The morphological data was then converted into a binary matrix

The morphological binary data was analysed using the Number Cruncher Statistical System (NCSS 2000) (Hintze, 1998). Dendrogrammes were constructed using the Unweighted Pair Group Method of Arithmetic Averages (UPGMA) in NCSS 2000. DNA extraction was done according to the method of Edwards et al. (1991). AFLP was performed according to Vos et al. (1995) using EcoRI and MseI restriction enzymes. Selective PCR reactions were done and resolved on an ABI Prism 310 Automated Capillary Sequencer. Primer combinations used were MseI-CAG and EcoRI-ACA, and MseI-CAG and EcoRI-AAC. DNA fingerprint analysis was done on a Macintosh (IMAC) computer using GeneScan 3.1 (Perkin-Elmer Corporation). Fragments were scored into a binary matrix as present (1) or absent (0). Then, the molecular binary data was analysed using NCSS 2000 (Hintze, 1998), and dendrogrammes were constructed using UPGMA.

RESULTS AND DISCUSSION

Spearman correlation using pair-wise comparison between morphologic and AFLP characterisation for genetic distance values, and linkage data showed strong correlation of $r=0.98$ for genetic distance values, and $r=0.82$ for linkage data for genotypes. Previous studies showed weak or no relationships between morphological and molecular genetic distances and clustering (Zacarias, 1997), which conflicts with the findings of this study. These results suggest that constant traits should form the basis for morphological characterisation, and that the data should be properly converted into binary matrix for cluster analysis. However, AFLP fingerprints give a more descriptive structure relationship than morphological characterisation, and hence can be used to remove duplicates in germplasm collections.

CONCLUSIONS AND RECOMMENDATIONS

Both methods clustered most genotypes in a similar fashion. There was a strong correlation between the morphological and AFLP genetic distance values ($r=0.98$) as well as the linkage data for genotypes ($r=0.82$). This study suggests that if constant traits form a basis of morphological characterisation, and the data correctly converted into binary matrix for cluster analysis, then the results are comparable to DNA molecular characterisation. Hence, morphological characterisation can be used to narrow down a collection. However AFLP fingerprints gave a more descriptive structure than morphological characterisation. AFLP can be a useful tool to isolate duplicates not able to be sorted out by morphological method. Thus, these two methods are complementary to each other.

REFERENCES Beeching, J.R., Marney, P., Gavalda, M.C., Noirot, M., Haysom, H.R., Hughes, M.A. and Charrier A., 1993. An assessment of genetic diversity within a collection of cassava (*Manihot esculenta* Crantz) germplasm using molecular markers. *Annals of Botany* 72(6): 515-520. Benesi, I.R.M, Mayo, C.C., Mkumbira, J., Mahungu, N.M. and Sauti, R.F.N., 1999. A proposal for the release of three promising cassava clones in Malawi. Presented to the Malawi ATCC, Lilongwe, Malawi. Brush, S., 1995. In situ conservation of landraces in centres of crop diversity. *Crop Sci.* 35: 346-354. Edwards, K., Johnstone, C. and Thompson, C., 1991. A simple and rapid method for the preparation of plant genomic DNA for PCR analysis. *Nucl. Acid Res.* 19: 1349. FAO, 2000. FAO statistical Database. Hintze, J.L., 1998. NCSS 2000 statistical system for Windows. Number Cruncher statistical Systems. Kaysville, Utah. Mahungu N.M. and Kanju, E., 1997. Cassava breeding manual. Reference document at the Regional workshop on cassava breeding, Dar-res- Salaam, Tanzania, 1997. Nweke, F.I., Dixon, A.G.O., Asiedu, R., and Folayan, S.A., 1994. Cassava varietal needs of farmers and potential production growth in Africa. COSCA working paper No. 10. IITA, Ibadan, Nigeria. Scott, G.J., Rosegrant, M.W. and Ringler, C., 2000. Global projections of root and tuber crops to the year 2020. *Food Policy* 25: 561-597. Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes M., Frijers, A., Pot, J., Peleman, J. Kuiper, M. and Zabeau, M., 1995. AFLP: a new technique for DNA fingerprinting. *Nucl. Acids Res.* 23(21): 4407-4414. Zacarias, A.M., 1997. Identification and genetic distance analysis of cassava (*Manihot esculenta* Crantz) cultivars using RAPD fingerprinting. MSc thesis, University of the Free State, Bloemfontein, South Africa.

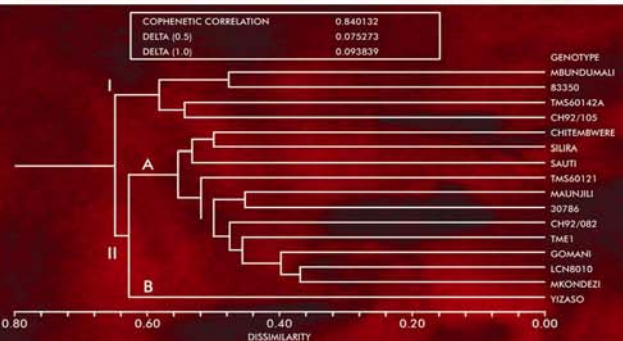


Figure 3: Phenetic dendrogram for morphological data of 16 elite Malawi cassava genotypes based on UPGMA from pair-wise comparisons employing Euclidean's coefficients of genetic distance.

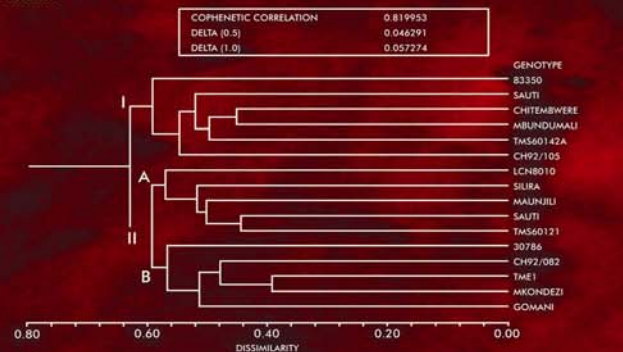


Figure 4: Phenetic dendrogram for AFLP data of 16 elite Malawi cassava genotypes based on UPGMA from pair-wise comparisons employing Euclidean's coefficients of genetic distance.