An Assessment of the Diversity in Global Cassava (*Manihot esculenta* Crantz) Genetic Resources based on Simple Sequence Repeat (SSR) Markers

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INTRODUCTION

Under the auspices of SP1 of the GCP, the genetic structure of a large and representative sample of cassava accessions held in germplasm banks at CIAT, EMBRAPA, and IITA was studied using simple sequence repeat markers. A sub-set of 3000 cassava accessions: 1500 from CIAT, 1000 from IITA, and 500 from EMBRAPA collections were selected based on criteria of location and key agronomic traits including drought tolerance, resistance to major pests and diseases, adaptation to different ecologies, etc. Data analyses was based on SSR marker data from 30 loci (212 alleles) 22 from CIAT and 8 from IITA. It include assessment of genetic structure using principal coordinate analysis (PCoA) based on individuals, cluster analysis based on countries and an estimation of genetic diversity and allelic richness. Preliminary results reveal a separation between accessions from Africa and the rest of the world confirming findings from several other studies that shows that global cassava germplasm diversity is structured by region with those from Africa showing the highest differentiation from those from the Neo-tropics. Sources of this genetic differentiation could be selection for adaptation to agro-ecologies, particularly disease, found in Africa, mutation, and even biased sampling.

MATERIALS AND METHODS

Assembling Cassava germplasm

Plant material from a subset of 3000 cassava accessions, out of the large group of over 10.000 varieties held in the three largest collections at CIAT, IITA and EMBRAPA, was used to isolate DNA at the respective centers (Figure 1). EMBRAPA accessions were extracted at CIAT from duplicate copies in the CIAT germplasm bank and finally, aliquots of each sample were gotten for the genotyping teams at IITA (Nairobi) and CIAT.

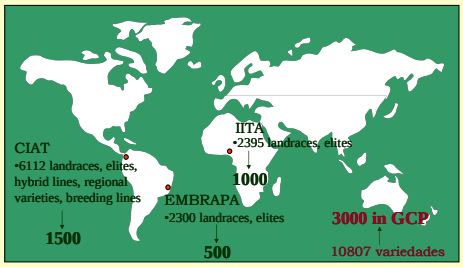

Figure 1. Building the "composite set" of 3000 accessions from germplas, collections around the world

The selection of the set of 3000 cassava accession was based on criteria that emphasizes location and key agronomic traits such as Drought tolerance, resistance to major pests and diseases, adaptation to different ecologies, etc. CIAT as lead institute compiled passport data, including the local names, source (Country/State/Province/Region/Village) and geographical position (Longitude, Latitude, Altitude), from all accessions into a data base that is accessible to the entire cassava research community (CIAT 2004).

Molecular markers set

Over 600 SSR markers exist for cassava and 67 were used to assess diversity in previous studies (Mba et al, 2001). 36 SSR markers, out of the 67, were selected to assess the diversity of global cassava genetic resources in GCP. The selected markers have clear and reproducible allele patterns as well as high PIC and those are representing the 18 linkage groups of cassava.

Genotyping

Genotyping was carried out using 36 SSR markers, 22 at CIAT following the silver staining protocol (Figure 2) and 14 at IITA using the ABI sequencer. Molecular weight information by locus and genotype has been compiled at CIAT, where alleles by locus per genotype as well as a binary matrix have been generated for data analysis.

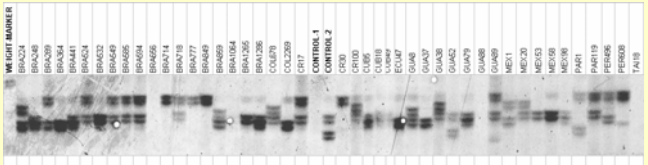

Figure 2. PAGE gel of PCR amplification of cassava accessions with SSR markers (SSRY4).

Data analysis

Data analysis was based on SSR markers data from 22 loci (212 alleles) evaluated at CIAT. The analysis includes assessment of genetic structure using principal coordinate analysis (PCoA) and multidimensional scaling (MDS) based on individuals and cluster analysis based on countries from a Jaccard's similarity matrix.

RESULTS

Preliminary results from MDS reveal a separation between accessions from Africa and the rest of the world (Figure 3) confirming findings from previous studies that shows how global cassava germplasm diversity is structured by region.

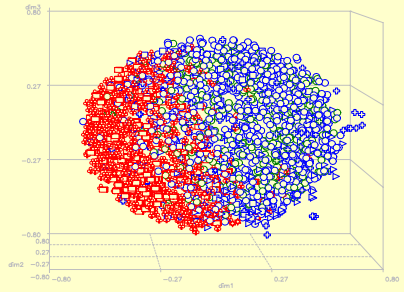

Figure 3. Multidimensional scaling plot from 2494 genotypes analyzed with 22 SSR markers. Accessions from IITA, CIAT and EMBRAPA are represented in red, blue and green respectively.

African accessions show the higher differentiation from those from the Neo-tropics (Figure 4). Sources of this genetic differentiation could be selection for adaptation to agro-ecologies, particularly disease, found in Africa, mutation, and even biased sampling.

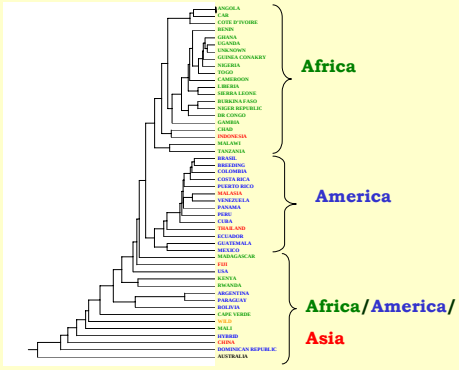

Figure 4. Cluster analysis based on 50 countries.

CONCLUSION

The results agree with previous diversity studies in Cassava where structure is explained by the accession's origin. The cluster composed by African/Asian/ American accessions could be explained by small sample size or recent introduction. Nigerian accessions have a broad diversity (data not shown) that could be explained by migration and selection for tolerance to drought and diseases. It is important to have complete information from the 36 SSR loci to better define the structural diversity.

REFERENCES

CIAT(Centro Internacional de Agricultura Tropical). 2001. Annual Report. Cali, Colombia.

R.E.C. Mba, P. Stephenson, K. Edwards, S. Melzer, J. Nkumbira, U. Gullberg, K. Apel, M. Gale, J. Tohme, M. Fregene. 2001. Simple sequence repeat (SSR) markers survey of the cassava (Manihot esculentaCrantz) genome: towards an SSR-based molecular genetic map of cassava. Theor Appl Genet (2001) 102:21–31