Simple Sequence Repeat (SSR) Assessment of Genetic Diversity of Local Cassava Varieties from Cuba

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INTRODUCTION

Cassava is an important crop of modern tropical economies and an attractive one for millions off resource poor farmers found in the tropics (Best and Henry 1994). Recently a second center of diversity have been postulated in Central America based on SSR markers (Monte et al. 2003), in addition to the one in Brazil (Olsen and Schaal 1999). However, the potential of diversity in the second center, particularly in the Caribbean is not well documented. Recently SSR markers have been utilized to study the diversity of cassava from different countries (Fregene et. al. 2003). SSR markers are particularly attractive to study genetic diversity due to their abundance in plant genomes, high levels of polymorphisms and adaptability to automation. These studies revealed a high amount of diversity in accessions from several neotropical countries, a low level of genetic differentiation between country samples, with the exception of a group of accessions from Guatemala, and sub-structure in diversity of accessions from some African countries.

SSR markers can contribute to a better understanding of genetic diversity present in a collection of local cassava varieties held in Cuba to permit a more rational conservation and use of diversity on the island. We present here preliminary results of SSR study of genetic diversity of cassava from Cuba compared to a sub-set of accessions from Africa, South and Central America.

MATERIALS AND METHOD

A total of 94 accessions were selected from a collection of cassava held at INIVIT in Cuba, selection criteria was the economic importance and origin in Cuba. A set of 54 clones from Africa and the Neotropics, 12 from Nigeria, 10 from Tanzania, 12 from Guatemala, and 20 from South America, representative of a large set of accessions from these countries used in previous SSR studies (Fregene et al 2003) were included for comparisons. A third set of 13 improved genotypes from CIAT with traits of agronomic interest were added. DNA from all accessions was obtained using the Dellaporta et al. method (1983). Concentration and quality of the DNA was checked by flourometry and agarose gel electrophoresis respectively. The DNA samples were diluted to a working concentration of 10ng/ul for subsequent PCR amplification.

PCR amplification, automated gel analysis and date collection were as descried by Fregene et al (2003) and Mba et al. (2003). Statistical analysis to be conducted include calculations of pair-wise genetic distance, based upon the proportion of shared alleles (PSA), using the computer microsat (Minch 1993, http://www.lotka.stanford.edu/microsat.html). Distances between the accessions will be subjected to principal component analysis (PCA) using JMP (SAS Institute 1995) to obtain a structure of relationship between the land races. Other analysis are estimation of parameters of genetic diversity and differentiation, calculated from the raw SSR allele data using the computer packages GENSURVEY (Vekeman et al 1997) and FSTAT (Goudet 1990).

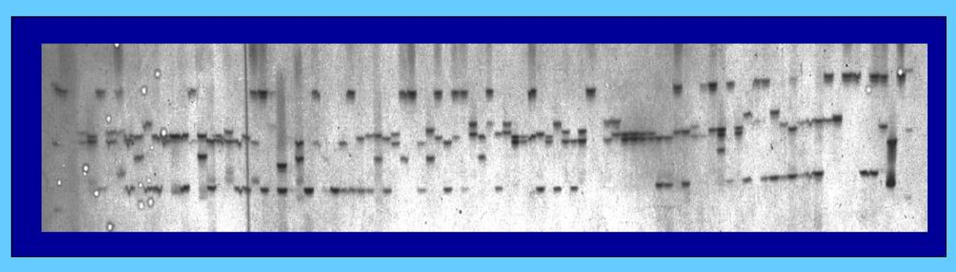

Figure 1 Polyacrilamide gel of a PCR amplification of cassava accessions from Cuba, Nigeria, Tanzania, Guatemala, and South America using the SSR primer SSRY51.

RESULTS AND DISCUSSION

A total of 34 SSR markers were analyzed in 86 accessions from Cuba, 10 each from Guatemala and Tanzania, 11 from Colombia and 16 from Nigeria. A high number of alleles, between 2 and 10, and a high level of polymorphisms have been observed in all SSR markers analyzed until date (Fig 1 and Table 1). Parameters of genetic diversity also revealed high levels of diversity in the Cuban collection, an average of 5.8 loci per locus and expected heterozygosity, corrected for sample sizes (Nei 1978), of 0.6351, average for all samples was 0.6292 ± 0.0120. The estimate of expected and observed heterozygosity was not significantly different between accessions from Latin America and Africa. Only 7.4% of the total heterozygosity can be attributed to differentiation between accessions by country. Estimates of genetic differentiation obtained from F_{ST} were low, ranging from 0.4 to 0.6. Relationships at the level of individual genotypes can be observed from the Principal Component Análysis (PCA) based on the genetic distance 1-proportion of shared alleles (1-PSA) shown in figure 2, all simples, and figure 3, only those from Cuba. The PCA reveals a sub structure in the Cuban materials as has been found in several African country studies. It is not clear at the moment if the substructure in Figure 3 represents graphically the relationships between casava accessions by geographic origin

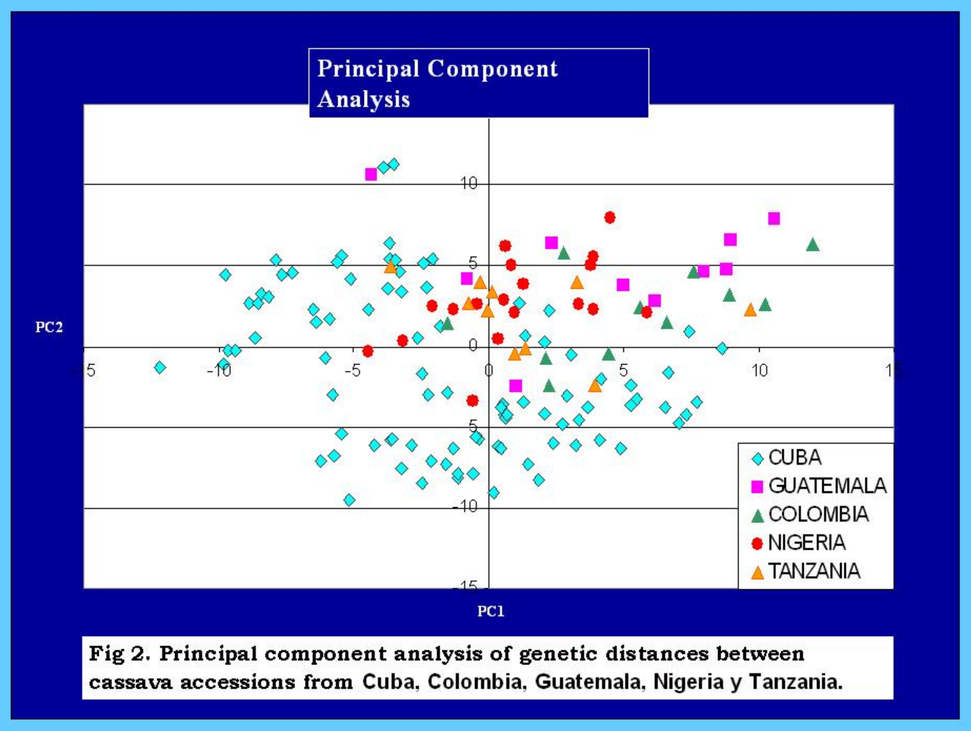

Fig 2. Principal component analysis of genetic distances between cassava accessions from Cuba, Colombia, Guatemala, Nigeria y Tanzania.

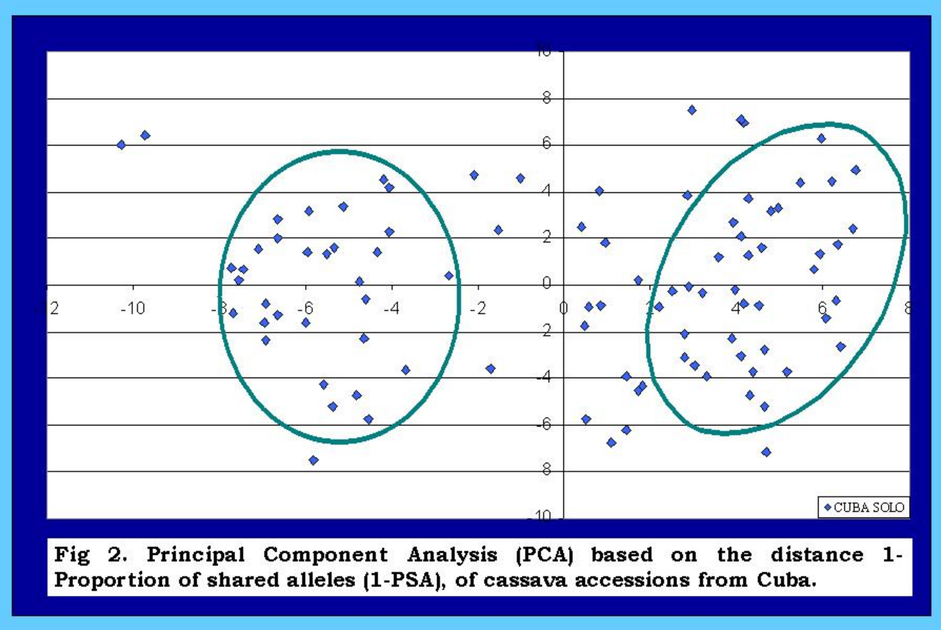

Fig 2. Principal Component Analysis (PCA) based on the distance 1-Proportion of shared alleles (1-PSA), of cassava accessions from Cuba.

Table 1.8 Intra-population and inter-population estimates of genetic diversity parameters of cassava land races from different agro-ecologies of Cuba, Guatemala, Colombia, Nigeria, Tanzania

Población	n	#loc	#loc_P	PLP	K	K_P	HO_p	HE_p	HEc_p
CUBA	86	34	34	100.0	5.8	5.8	0.6016	0.6314	0.6351
GUATEMALA	10	34	34	100.0	4.2	4.2	0.5556	0.6063	0.6385
COLOMBIA	11	34	33	97.1	4.5	4.6	0.5675	0.6087	0.6396
NIGERIA	16	34	33	97.1	4.5	4.6	0.5885	0.5949	0.6136
TANZANIA	10	34	31	91.2	4.2	4.5	0.6459	0.5869	0.6190
Media	5 Poblac.			97.06	4.64	4.72	0.5918	0.6057	0.6292
Desv.Stand.				3.60	0.65	0.61	0.0351	0.0169	0.0120

Ho: observed heterozygosity He: Average gene diversity Hec_p: Average gene heterozygosity corrected for small samples sizes

CONCLUSIONS AND ONGOING WORK

SSR diversity of cassava of 94 accessions from Cuba was studied and results revealed a sub structure among the Cuban materials Low genetic differentiation was observed between the Cuban and accessions from other parts of Africa and Latin American, suggesting exchange of genetic material between the island and the rest of the cassava growing world. Future perspective include trying to trace the historical antecedents of these accessions studies so as to be able to elucidate the sub structures observed

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