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

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INTRODUCTION

Two primary centers of diversity, one in South America and the other in Meso-America have been postulated for the genus *Manihot* (Roger and Appan 1973). Although several studies have demonstrated a likely South American origin for the cultivar (Allem, 1994; Fregene et.al 1994; Roa et al. 1997; Olsen and Schaal 1999), the diversity of cassava and its wild relatives in Memo- America is great enough to suggest a second center in Meso-America. Besides, the potential of Meso-American diversity in cassava improvement has not been properly assessed. Three recent studies of genetic diversity in land races from South America and Meso-America (Chavariagga et. al. 1999; Fregene et. al. 2002; Raji et. al. unpublished data) have revealed unique alleles in land races from Guatemala at a frequency high enough to suggest a Meso American center of cassava diversity. The results of the three studies were based upon 6, 4, and 13 Guatemalan land races. The small sample size of the previous study could distort the allele frequencies and lead to wrong conclusions. A larger collection and SSR characterization of land races from Guatemala was therefore planned to confirm preliminary data of a Meso-American center of diversity and to secure the largely untapped diversity in Guatemala before it becomes extinct. In addition, a selection from the Guatemalan collection will be crossed to CIAT elite parents to evaluate the utility of the Meso-American diversity in cassava breeding.

The present study was to confirm the high genetic differentiation between cassava land races from Guatemala and Nigeria, Brazil, and Colombia. If the uniqueness of the Guatemalan germplasm is confirmed, genetic crosses to CIAT's elite breeding lines will be made to test hybrid vigor and delineate heterotic pools. Plant materials are a collection of cassava from all over Guatemala and a representative group used in previous studies from Nigeria, Colombia and Brazil to confirm earlier results. It is hoped that results of the uniqueness and the utility of the Guatemalan germplasm will give collection and conservation of this germplasm in regions of Meso-America high priority (Azurdia and Gomez 2002)

MATERIALS AND METHOD

A collection of cassava land races was carried out all over Guatemala in May last year (Azurdia and Gomez 2002). A total of 128 accessions were collected in the departments of Baja Verapaz, Quiche, Huehuetenango, Alta Verapaz, San Marcos, Escuintla y Santa in Guatemala. See Guatemalan study on the MOLCAS web site (http://www.ciat.cgiar.org/Molcas) for names of accessions. For comparison with results of previous studies, DNA from 6, 11 and 12 cassava land races from Nigeria, Colombia y Brazil respectively were included, making 4 samples for the analysis of genetic diversity and differentiation. DNA from the Guatemalan accessions was isolated at the Facultad de Agronomia, Universidad de San Carlos de Guatemala using a micro-prep protocol of the Dellarporta (1983) methodology and transferred to CIAT. DNA from the other accessions was obtained from previous studies at CIAT.

A set of 36 SSR markers, carefully chosen to represent a broad coverage of the cassava genome with moderate to high polymorphism information content (PIC) and robust amplification, were used in this study. SSR markers, PCR amplification, polyacrylamide gel electrophoresis, and silver staining used in this study have been described elsewhere (Fregene et al 2002). The allele data was captured using the program "Quantity One" (Bio-Rad Inc) and entered directly into EXCEL (Microsoft Inc) for statistical analysis. Statistical analysis on the raw SSR data include: genetic distance analysis using a of a distance matrix based upon 1-proportion of shared alleles (1-PSA), principal component analysis (PCA) and cluster analysis (UPGMA) of the distance matrix, and parameters of genetic diversity and differentiation.

RESULTS AND DISCUSSION

A total of 33 SSR markers were analyzed in the 128 accessions from Guatemala that includes an accession of the wild relative *M.aesculifolia*. Unique alleles were observed in the accessions from Guatemala for the markers SSRY 12 (0.14), SSRY 20(0.383), SSRY 34(0.006), SSRY 38(0.063), SSRY 51(0.1), SSRY 59(0.014), SSRY 63(0.014), SSRY 69(0.023), SSRY 82(0.007), SSRY 103(0.24), SSRY 108(0.043), SSRY 135(0.13) y SSRY 147(0.013). In parenthesis are the frequencies of the observed alleles. The first and second principal components of the PCA, based on upon the genetic distance 1-proportion of shared alleles, are shown in Fig 8.3. Accessions from Guatemala form two groups, one that clusters along with land races from Brazil, Nigeria and Colombia in a broad group and a second group that is clusters separately. The only sample from *M. aesculifolia* is located far away from both clusters. The results observed confirms previous observation of a high genetic differentiation of between certain groups of cassava land races from Guatemala and these from other parts of Latin America and Africa (Fregene et al. 2003)

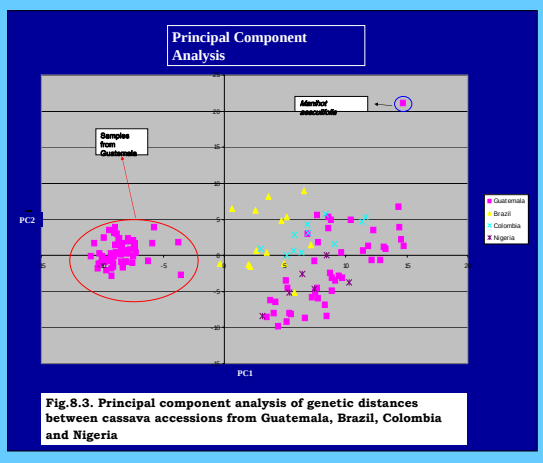

Fig.8.3. Principal component analysis of genetic distances between cassava accessions from Guatemala, Brazil, Colombia and Nigeria

Assessment of genetic diversity was based on samples of cassava land races from the 4 countries, with an addition that accessions from Guatemala were divided into two groups G1 and G2 based on clustering from PCA of genetic distances and UPGMA of F_{ST} data. Table 8.8 summarizes the parameters of genetic diversity observed for accessions from the 5 samples. Genetic diversity, as assessed by the average gene diversity (H_E) was high in the accessions analyzed 0.5422 ±0.2468. The population with the highest diversity was Colombia followed by the cluster G2 and that with the lowest was the cluster of the Guatemalan accessions clustered separately from other accessions. Average number of alleles was 3.8+/- 0.0358 for all accessions. Average number of alleles per locus was highest in the cluster G2 of Guatemalan land races, 5.2, and the lowest in the Nigerian land races, 2.9.

Table 8.8 Intra-population and inter-population estimates of genetic diversity parameters of cassava land races from different agro-ecologies of Guatemala, Brazil, Colombia, Nigeria

Pop .	n	No. of Loc	No. of loci Pol.	Percent of Pol. Loc	Average No. of alleles/Loc.	Average No. of allles/Loc. Pol.	HO	HE	Hec.p
G1	24	33	28	84.8	2.8	3	0.6273	0.41	0.419
G2	74	33	33	100	5.2	5.2	0.5895	0.6066	0.6107
BRA	12	33	32	97	4	4.1	0.5562	0.5745	0.6013
COL	8	33	33	100	4.1	4.1	0.5912	0.6111	0.6555
NIG	4	33	31	93.9	2.9	3.1	0.648	0.5085	0.6067
	mean	**33**	**31.4**	**95.15**	**3.8**	**3.87**	**0.6024**	**0.5422**	**0.5786**
	std	**6.28**	**0.98**	**0.91**	**0.0358**	**0.0845**	**0.0918**	**0.2468**	

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

A UPGMA cluster analysis of the genetic distance data also produced 2 clusters of the Guatemalan accessions similar to that found with the PCA (data not shown). In addition, 2 sub groups were found within the group G1 that clustered away from the majority of accessions. A UPGMA of a pair-wise analysis of genetic differentiation (F_{ST}) again confirmed the separation of a group of accessions from Guatemala (Fig 8.4) as observed with the PCA and UPGMA analysis of genetic distances. The geographical distribution of accessions in cluster G1 can be observed in Figure 8.5. The distribution of accessions closely mirrors the distribution of 2 wild *Manihot* species in Gutemala, namely *Manihot rhomboide* and *Manihot aesculifolia* (Fig. 8.5). The majority of accessions in sub group A are found in western Guatemala and they overlap, as regards geographical origin, with *Manihot rhomboide*. On the other hand, genotypes from sub group B are found mostly in the Eastern part of the country together with natural populations of *Manihot aesculifolia*.

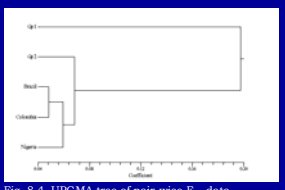

Fig. 8.4. UPGMA tree of pair-wise F_{ST} data calculated between samples from the four different countries

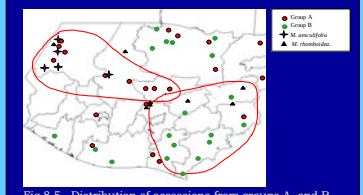

Fig 8.5. Distribution of accessions from groups A and B, of G1 and *Manihot aesculifolia* and *Mahihot rhomboide* in Guatemala

The origins of highly differentiated samples of cassava germplasm from Guatemala can be explained by independent domestication events in populations of different *Manihot* species that yet exist or are now extinct. They can also be explained by an introgression from *Manihot* species in certain regions that overlap in geographical spread with cassava. Cassava is an allogamous crop and natural cross pollinate between cassava and populations of wild *Manihot* species has been demonstrated Wanyera et al (1993).

CONCLUSIONS AND ONGOING WORK

A previous study of the assessment of genetic diversity of cassava land races in 14 South and Central American and African countries revealed a number of unique alleles in accessions from Guatemala and suggests a second center of diversity in Guatemala (Fregene et al 2003). Meso America is a center of diversity for many other food crops including common beans, maize, amongst others. This study shows unique alleles from Guatemala for a higher number of SSR markers and provides additional evidence for possible independent domestication of cassava in Meso America. However, an introgression with *Manihot* species that overlap with the geographical origins of these accessions makes it impossible to rule out introgression with these species. Further studies are required to clarify which is the most likely scenario via the collection and characterization of wild *Manihot* species the eastern and western parts of Guatemala. Additional activities include a diallel cross of theses excellent land races from Guatemala and other regions.

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