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Genetics of the genus *Acrocomia (Palmae)*. III Microgeographical genetic variability in isozyme frequencies

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ABSTRACT

In this study we provide an estimate of genetic variability in ten samples of *Acrocomia aculeata* (*macaúba palm*) collected across Belem Brasília highway (BR-153), Brazil, in the North of the State of Goias, between km 165 and km 231. Twelve presumptive loci were analysed using electrophoretic techniques and the allelic frequencies were calculated for each sample through the isozyme phenotypes. The observed heterozygosity varied from 0.042 to 0.667 with a mean of 0.182, while the calculated heterozygosity ranged from 0.087 to 0.499 with a mean of 0.375. Nei's genetic distance ranged between 0.020 and 0.447, with a mean of 0.125. These results show that the values of calculated heterozygosity are always higher than the values of observed heterozygosity, suggesting a deviation from Hardy-Weinberg expectations. They also indicate that there is free change of genes among these subpopulations which is common in the studied populations of *A. aculeata*. The genetic variability is similar to that which is found in populations studies of one species. The genetic variability in *A. aculeata* does not seem to be structure spaciously or correlated with the increase of the geographical distance.

Key words: Allelic frequency, Hardy-Weinberg's law, Macaúba palm.

INTRODUCTION

Electrophoretic surveys are frequently used to describe the genetic variation in natural plant populations (Hamrick *et al.*, 1979; Hamrick and Loveless, 1986; Buckley *et al.*, 1988). Our knowledge of genetic variation within and between populations is insufficient for the temperate zone trees and is poor for numerous tropical species, expecially for oil palm species. There are many important oil palm species in Brazil. One of them, *Acrocomia aculeata* (macaúba), has a great potential to produce different kinds of oil. Under uncontrolled conditions the *Acrocomia* palm oil yield is much larger than that of dendê (*Elaies guineensis*) (Markley, 1956; Wandeck and Justo, 1983). However, virtually nothing is known about the amount and patterns of genetic variability and about the relationships within and between populations of *Acrocomia aculeata*, a palm tree which occupies all over Brazil.

Here we provide an estimate of the genetic variation within a population of *A. aculeata*. Ten subpopulation samples were obtained in nearby areas across Belem-Brasília highway (BR-153), in the North of the State of Goias, between km 165 and km 231, where exist a great demographic concentration, constituing a well sampled microgeographical region. The purposes of this preliminary analysis were (I) to determine the levels of the genetic substructure within this geographical area, (II) to stablish the genetic relationships among the different collected materials and (III) to compare the apportionment of genetic variation between this population and other populations of the species.

MATERIALS AND METHODS

Electrophoresis was conducted on seedlings obtained from tissue culture of embryos derived from macáúba seeds. Macaúba fruits were collected within ten locations across Belem-Brasília highway (BR-153), in a microgeographical region, from km 165 to km 131. A total of 216 seedlings were analyzed from 18 trees in all the samples included in this study. The macaúba fruits collected in this research represent open-pollinated half-sib families.

The following polymorphic enzyme systems were analyzed: Phosphoglucoisomerase, acid phosphatase,

TABLE 1

Allelic frequencies at 12 presumptive loci, with two alleles each, for 10 collection locations of *Acrocomia aculeata*, from a microgeographical area

Sample	Locus																							
	Pgi-1		Pgi-2		Pgi-3		Acp-1		Per-1		Per-2		Per-3		Mdh-1		Mdh-2		Mdh-3		Est-1		Est-2	
	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S
1	.500	.500	.471	.529	.563	.437	.795	.205	.283	.717	.304	.696	.283	.717	.476	.524	.229	.771	.792	.208	.667	.333	.864	.136
2	.364	.636	.342	.658	.450	.550	.889	.111	.667	.333	.667	.333	.667	.333	.361	.639	.531	.469	.300	.700	.795	.205	.370	.630
3	.000	1.000	.000	1.000	.136	.864	.688	.312	.500	.500	.455	.545	.469	.531	.191	.809	.393	.607	.468	.532	.441	.559	.159	.841
4	.625	.375	.500	.500	.333	.667	.750	.250	.545	.455	.364	.636	.450	.550	.438	.562	.174	.826	.583	.417	.396	.604	.521	.479
5	.500	.500	.210	.790	.778	.222	.803	.197	.120	.880	.140	.860	.160	.840	.419	.581	.357	.643	.500	.500	1.000	.000	.542	.458
6	.500	.500	.647	.353	.886	.114	.864	.136	.389	.611	.500	.500	.444	.556	.405	.595	.053	.947	.600	.400	.955	.045	.295	.705
7	.667	.333	.250	.750	.182	.818	.818	.182	.000	1.000	.000	1.000	.000	1.000	....	....	....	....	.682	.318	1.000	.000	.792	.208
8	....	....	....	....	....	....	.792	.208	.556	.444	.556	.444	.444	.556	.273	.727	.048	.952	.614	.386	.316	.684	.500	.500
9	.469	.531	.500	.500	.429	.571	.813	.187	.056	.944	.056	.944	.056	.944	.409	.591	.583	.417	.400	.600	.846	.154	.735	.265
10	.458	.542	.227	.773	.300	.700	.727	.273	.250	.750	.433	.567	.500	.500	.500	.500	.250	.750	.500	.500	1.000	.000	.354	.646

F = FAST – moving band (allele 2).
S = SLOW – moving band (allele 1).
.... = missing data

peroxidase, malate dehydrogenase and esterase. Few other loci were also polymorphic but proved not to be interpretable and were dropped from the analysis. The methods of tissue extraction and electrophoresis are described in detail by MORETZSOHN *et al.*, (1992).

Due to the long generation cycle of macaúba, (approximately seven years) it was not possible, to carry out progeny tests and thus genetic interpretations are based on the patterns of enzyme polymorphisms from natural populations. Plant genotypes were inferred from isozyme phenotypes including 12 presumptive loci. The loci analyzed are consistent with a simple genetic model based on an interpretation of monomeric enzymes.

Allelic frequencies were calculated for individuals in the different localities. Gene and genotype frequencies were used to calculate the average observed heterozygosity per locus, the average calculated heterozygosity and the Nei's genetic distance (NEI, 1972, 1987). UPGMA phenogram (SNEATH and SOKAL, 1973) was constructed to analyze the pattern of population relatedness. Mantel's nonparametric Z test was employed to determine whether genetic distance between populations was correlated with geographic distance.

RESULTS

Interpretations of enzyme phenotypes are based on patterns of variability in ten samples obtained in a microgeographical area 66 kilometers in length. The 12 putative enzyme loci assayed were represented by two alleles each (the slower-migrating band is designated allele 1, and the faster-migrating one as allele 2). The frequencies of the alleles 2 are presented in Table 1. The genotypic frequencies are given in Table 2 and the observed and calculated heterozygosities are shown in Table 3.

Population structure deviates from HARDY-WEINBERG expectations, with the observed heterozygosity lower than the calculated heterozygosity in almost all samples analysed. In this population of ten samples, both alleles in most of the loci, deviate in the direction of excess homozygosity and there is a majority of the slower-migrating bands (alleles 1). The observed heterozygosity is higher than the calculated heterozygosity only in sample 2 for Acp-1, in sample 4 for Pgi-2 and Est-1, in sample 5 for Pgi-2 and Est-2, in sample 7 for Pgi-1 and Pgi-2, and in sample 8 for Est-1.

Based on the 12 polymorphic loci in Table 1 for which allele frequencies were available for the ten populations, the values of Nei's genetic distances (D) ranged from 0.020 to 0.447, with a mean of 0.125 (Table 4), as expected for conspecific populations (NEI, 1976). The UPGMA phenogram obtained from the Nei's genetic distances (Fig. 1) reveals that there was no relationship between D and the geographical distance, the different samples being connected each other at random.

TABLE 2

Genotypic frequencies for 12 loci in ten collection locations of *Acrocomia aculeata*

Locus	Genotypes[a]			
	No of seedlings	1/1	1/2	2/2
Pgi-1	152	0.401	0.283	0.316
Pgi-2	122	0.443	0.303	0.254
Pgi-3	118	0.500	0.017	0.483
Acp-1	164	0.128	0.134	0.738
Mdh-1	133	0.632	0.158	0.210
Mdh-2	129	0.620	0.163	0.217
Mdh-3	128	0.656	0.125	0.219
Est-1	223	0.404	0.448	0.148
Est-2	216	0.685	0.171	0.144
Per-1	173	0.329	0.266	0.405
Per-2	197	0.228	0.122	0.650
Per-3	199	0.462	0.131	0.407

[a] 1 = allele 1 (slow-moving band).
2 = allele 2 (fast-moving band).

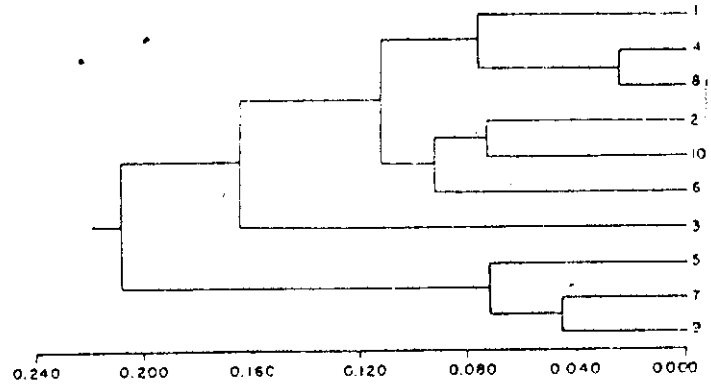

FIGURE 1 - Unweighted pair-group arithmetic average (UPGMA) cluster analysis performed using Nei's genetic distances, from 12 presumptive loci analysed in 10 collection locations.

DISCUSSION

The population of *Acrocomia aculeata* analysed here showed high genetic variation, with several polymorphic loci. Preliminary data for tropical tree species, but not palms, also indicate high genetic variation, though many species seem to possess

TABLE 3

Observed and calculated heterozygority to 12 presumptive loci in 10 collection location of *Acrocomia aculeata*

Sample	Pgi-1	Pgi-2	Pgi-3	Acp-1	Mdh-1	Mdh-2	Mdh-3	Est-1	Est-2	Per-1	Per-2	Per-3
Calculated heterozygosity												
1	0.500	0.499	0.492	0.325	0.405	0.423	0.405	0.496	0.297	0.330	0.444	0.236
1	0.500	0.499	0.492	0.325	0.405	0.423	0.405	0.496	0.297	0.330	0.444	0.236
2	0.463	0.450	0.495	0.198	0.444	0.444	0.444	0.461	0.498	0.420	0.325	0.463
3	0.000	0.000	0.236	0.430	0.500	0.496	0.498	0.309	0.477	0.498	0.493	0.272
4	0.467	0.500	0.444	0.375	0.496	0.463	0.495	0.492	0.287	0.486	0.478	0.499
5	0.500	0.332	0.346	0.316	0.211	0.241	0.269	0.487	0.459	0.500	0.000	0.497
6	0.500	0.457	0.201	0.236	0.475	0.500	0.494	0.482	0.100	0.480	0.087	0.423
7	0.444	0.375	0.297	0.297	0.000	0.000	0.000	----	----	0.434	0.000	0.330
8	----	----	----	0.330	0.494	0.494	0.494	0.397	0.093	0.469	0.435	0.500
9	0.498	0.500	0.490	0.305	0.105	0.153	0.105	0.483	0.486	0.480	0.260	0.367
10	0.497	0.351	0.420	0.397	0.375	0.491	0.500	0.500	0.375	0.500	0.500	0.457
Observed heterozygosity												
1	0.167	0.375	0.000	0.136	0.130	0.174	0.130	0.469	0.182	0.083	0.000	0.250
2	0.182	0.053	0.000	0.222	0.000	0.000	0.000	0.389	0.138	0.200	0.046	0.046
3	0.000	0.000	0.091	0.125	0.500	0.364	0.188	0.392	0.286	0.419	0.353	0.125
4	0.417	1.000	0.000	0.136	0.182	0.364	0.100	0.542	0.174	0.250	0.208	0.042
5	0.091	0.105	0.000	0.152	0.000	0.400	0.080	0.452	0.143	0.417	0.000	0.083
6	0.455	0.471	0.046	0.091	0.111	0.111	0.222	0.429	0.105	0.400	0.000	0.133
7	0.667	0.417	0.000	0.167	0.000	0.000	0.000	0.583	0.000	0.250	0.000	0.083
8	----	----	----	0.083	0.333	0.333	0.167	0.460	0.086	0.133	0.292	0.083
9	0.438	0.000	0.000	0.125	0.000	0.000	0.000	0.273	0.167	0.000	0.000	0.227
10	0.250	0.091	0.000	0.182	0.214	0.200	0.200	0.455	0.250	0.250	0.000	0.208

---- = missing data.

TABLE 4

Matrix of Nei's genetic distances for 10 collection locations of *Acrocomia aculeata*, in a microgeographical area

	1	2	3	4	5	6	7	8	9	10
1	0.000									
2	0.170	0.000								
3	0.339	0.205	0.000							
4	0.061	0.106	0.138	0.000						
5	0.068	0.164	0.224	0.149	0.000					
6	0.099	0.117	0.256	0.114	0.089	0.000				
7	0.082	0.311	0.314	0.178	0.074	0.241	0.000			
8	0.101	0.135	0.060	0.017	0.202	0.095	0.307	0.000		
9	0.071	0.195	0.243	0.143	0.046	0.173	0.036	0.259	0.000	
10	0.097	0.075	0.116	0.092	0.069	0.089	0.113	0.142	0.108	0.000

1 to 10 = differens.

low levels of genetic polymorphism (HAMRICK and LOVELESS, 1986).

The high level of variation within this population of *A. aculeata* is not surprising because of the high outcrossing rate in this species, about 85%. Considering the diverse pollen and seed dispersal mechanisms, one would expect that diversified population structures occur in tropical forest trees. Moreover, the free exchange of genes would account for the occurence of diversified populations without any relationship to the geographical distribution.

The results also show that there was a consistent excess of homozygosity, as obtained for some loci in Brazil nut by BUCKLEY *et al.*, (1988), suggesting that inbreeding would be relatively frequent among populations of *A. aculeata* or that some force affects the relationship between gene and genotypic frequencies. In the latter case it is likely that some sort of disruptive selection occurred, in which the direction and the intensity of selection depend on the degree of the alterations of the environment.

Our results may have great implications in the preservation and utilization of the germplasm of *Acrocomia* palms in Brazil. If these and other results on isolated geographical populations (LOPES *et al.*, 1991) will be confirmed by further analysis, a great portion of the genetic variability is preserved and may be obtained from one or few populations. It is obvious that such populations must be very large because it appears that the large amount of genetic variation in *Acrocomia* populations must be maintained by extensive gene flow and bonds of mating over a large area.

All the results on genetic variability in *Acrocomia* obtained through the isoenzymatic polymorphism confirm the taxonomic revision done by LLERAS and CORADIN (1990). These authors believe that only one species, *A. aculeata* occupy about the entire country of Brazil, with the exception of a small area in the north of Minas Gerais State, where it can be found the species *A. hasleri*.

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