

ENZYMATIC VARIABILITY IN NATURAL POPULATIONS OF *Hevea brasiliensis* (Willd. ex ADR. de Juss.) Müell. Arg.

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SUMMARY

In order to infer on the genetic structure and to quantify the genetic variability between 2 natural populations of rubber trees (*Hevea brasiliensis* (willd.) Muell. Arg.) through isoenzymic characterization rubber tree seeds were collected in the State of Acre. Electrophoretic analyses were carried out in starch gel with fresh leaflets extracts for 4 enzymatic loci (MDH-1, LAP-1, LAP 2, and SKDH). The assessed variability parameters present high genetic variation levels for both rubber tree populations ($H_T = 0.3356$). This value is superior to other average tropical arboreal species. The largest part of the total variability is found within the populations (99.85%). The estimates for the outcrossing rate ($t = 64,46\%$ e $64,15\%$) of rubber tree in natural populations indicate a tendency of that species to belong to the intermediate group of plants concerning on mating system.

Key words: Rubber tree, natural populations, eletrophoresis technique, outcrossing rate, in breeding coefficient.

INTRODUCTION

The great natural genetic variability of *Hevea brasiliensis*, the rubber tree, is one of the main treasures available to those interested in expanding the potential of domesticating this specie (Gonçalves *et al.* 1983), especially in the traditional regions, where despite the tradition of cultivating rubber trees, the climatic conditions are excellent for the flourishing of diseases.

However, considering its potential, the rational use of the genetic variability of natural populations has been quite modest, and totally directed towards obtaining clones resistant to diseases (Brazil 1971).

As we increase our knowledge about the form of organization, maintenance and distribution of the genetic variability of tropical species, credence is gained for the hypothesis that, the rational cultivation of a specie in a tropical environment must live in equilibrium with the biotic factors of the ecosystem, otherwise it is doomed to fail. Besides this, the knowledge of the natural populations will obviously allow greater exploitation of existing genetic variability, both for direct use in genetic improvement and as a means of orienting the collection, sampling and preservation of native rubber tree germ plasm.

Through iso-enzymatic characterization the objectives are to estimate some genetic parameters of the population and to quantify the existing genetic variability of two native rubber tree populations.

MATERIAL AND METHODS

The material utilized consists of plants originating from seeds collected from two different natural populations of rubber trees, located in an area of EMBRAPA-UEPAE - Rio Branco and the Catauba Forest Reserve which belongs to the Federal University of Acre (UFAC), at KM 14 and KM 22, respectively, of the BR 364 Highway, in the state of Acre.

Electrophoretic analyses were carried out in 26 and 27 plant families, respectively for population 1 and 2, with a variable number between 14 and 24 plants/family at the Biological and Genetic Reproduction Laboratory of DCF/ESALQ in Piracicaba - Sao Paulo.

The enzymatic systems utilized were malate dehydrogenase (MDH) - EC 1.1.1.37, shiguimato dehydrogenase (8KDH) - EC 1.1.1.25 and leucine aminopeptidase (LAP) - EC 3.4.11.1 (Lebrun and Chevallier 1988) (Table 1).

TABLE 1. Enzymatic system, abbreviations, plugs, allo-enzymes and relative mobility.

ENZYME	ABBREVIATIONS	PLUGS			ALLOENZYME	RM
		ELECTRODE GEL COLOUR				
Malato desidrogenase EC 1.1.1.37	MDH-1	A	B	C	1	100
					2	90
					3	80
Shiquimato desidrogenase EC 1.1.1.25	SKDH	A	B	C	1	100
					2	115
					3	98
Leucina aminopeptidase EC 3.4.11.1	LAP-1	A	B	D	4	76
					5	56
					1	100
					2	107
					3	116
	LAP-2	A	B	D	4	93
					5	87
					1	100
					2	113
					3	91
			4	76		

* - A: Tris citrato pH 6,6; B: Histidine 0,05M pH 6,0; C: Tris HCl pH 8,5 e D: Tris Maleato pH 5,4 (Lebrun & Chevallier 1988).

For each allele identified a variance analysis based on the unbalanced hierarchical model (Anderson & Brancroft, 1952; Weir 1990) was carried out. From the estimates of the variance components calculations for: the mean coefficient of endogamy for all of the plants from the two populations (F); the rate of crossfertilization of the specie (t) and the genetic distance between populations (e_2) were realized (Weir, 1990; Vencovsky 1992).

The computer program BIOSYS-1 (Swofford 1989) which provides estimates of the F statistics of Wright (1978) and the genetic distance according to Nei (1972) between populations was used for the analysis of data related to the loss in family structure for each population. The genetic diversity between families was also estimated using the parameters established by Nei (1973).

The rates of cross-fertilization and the endogamy coefficients within populations were also estimated through expected (A_0) and observed frequencies for heterozygote, according to Li (1955) and Weir (1990).

Two loci (LAP-1 and LAP-1) were identified in the leucine aminopeptidase system (LAP). Five alleles were identified in LAP-1, which according to the norm were denominated allele 1 for the most common and alleles 2 and 3 for those that respectively represented relative migrations IRM'S superior to 1, and alleles 4 and 5 for those presenting RM's inferior to 1 (Table 1). In the loci of LAP-2, of slower migration, four alleles were identified, with only allele 2 representing RM superior to one, the rest being inferior. Only one locus was identified in the gKDH system, with five alleles. Allele 2 had an RM superior to 1.

The MDH system showed a band pattern which was difficult to interpret for all of its loci. The fastest locus in the system was identified, which had three alleles. Allele 3, the slowest, overlapped with the fastest allele of possibly another locus.

The monomeric structure of the enzymes for SKDH and LAP agree with the results obtained by Irca 11986), Chevallier *et al.* (1984) and Chevallier (1988) but don't agree as to the number of alleles

TABLE 2. Estimates of the endogamy coefficient (f and f) and the cross-fertilization rate (t) for 17 alleles in four locus of the two natural populations of rubber trees¹.

LOCI	LI (1955)*		WEIR (1990)	
	f	t	f	t
LAP-1	0,1644 (0,0288)	0,7176 (0,0424)	0,1664 -	0,7147 -
LAP-2	0,2179 (0,0412)	0,6422 (0,0577)	0,2099 -	0,6530 -
SKDH	0,2481 (0,0257)	0,6024 (0,0173)	0,2488 -	0,6008 -
MDH-1	0,1956 (0,0755)	0,6728 (0,1058)	0,2535 -	0,6502 -
MÉDIA	0,2184	0,6415	0,2181	0,6446

¹Adapted from Paiva *et al.* (1992a e 1992b).

²Values in parenthesis correspond to the standard deviation.

per locus. In both systems, the number of alleles per locus were above that of previous analyses, thereby demonstrating greater genetic variability in these populations.

The average cross fertilization rate for rubber trees in natural populations was 64.465 and the average coefficient of endogamy was 21.815 (Table 2), estimated using Weir's (1990) methodology. Estimating these same coefficients using Li's (1955) methodology gave respective results of 64.15% and 21.845.

For geneticists working with the rubber tree, the rate of natural fertilization of the species was always an unknown factor, despite its great importance for the definition of improvement strategies and for understanding the genetic structure of the populations.

Using an multi-loci methodology, O'Malley *et al.* (1988) estimated a fertilization rate for "t" = 0.85 +/- 0.03 for the Brazil nut tree (*Bertholletia excelsa*), pointing out that allogamy is predominant in this species; although it did not rule out the possibility of low levels of endogamy occurring.

It can be verified that the average rate of endogamy for the rubber tree in natural populations has always been above 20%. With this result, one cannot state that endogamy has originated from the self-fertilization of the plants, *i.e.* with a cross fertilization rate around 64%, then the rate of self-fertilization would be 36%, keeping in mind that endogamy may originate from the fertilization of related plants.

Simmonds (1989) reports that studies on cross-pollination in rubber trees has apparently not been well understood, due to the occurrence of dwarf plants, possibly caused by self-fertilization of plants in seed gardens of good progenies. This author points out that the self-fertilization rate varies between 16 - 28%. He furthermore reports that the estimated rate of self-fertilization of the clone PB 5/51 at Prang Besar (a private rubber tree plantation firm in Malaysia) was 22%.

The estimated parameters for variability show high levels of genetic variation for both populations in the set (Ht = 0.3356) (Table 3) and that the greatest proportion of variability was found within the population (99.85%).

TABLE 3. Total genetic diversity (H_t) within the population (H_s), between populations (D_{ST}) and the proportion of diversity in relation to the total (G_{ST}) for four loci in two natural populations of rubber trees.

LOCOS	N*	H_T	H_S	G_{ST}	D_{ST}
LAP-1	1020	0,3109	0,3096	0,0042	0,0013
LAP-2	962	0,2211	0,2206	0,0023	0,0005
SKDH	853	0,6253	0,6239	0,0022	0,0014
MDH-1	976	0,0726	0,0712	0,0193	0,0014
MÉDIA	3811	0,3356	0,3351	0,0003	0,0001

* = total number of plants analyzed in the two populations.

This greater proportion observed for variability within rubber tree populations indicates that collection of genetic material from rubber trees for conservation of genetic resources may be directed towards one or only a few populations, within a certain collecting region, and of a larger number of individuals per population. Using this procedure, one can collect more genetic material representative of the specie at a lower cost.

Chevallier (1988) had already pointed out that the high rate of variation of the rubber tree in its site of origin, would be in accordance with the perennial nature of the species, the presumed mode of cross-fertilization as a form of reproduction and the manner of seed dispersion by animals or by river, are factors acting to maintain the high levels of variation within the populations.

This is why Gonsalves (1981) observed that the size of the rubber trees in native stands in the state of Acre, located in the terra firme (areas not subject to flooding) were much larger than the rubber trees of the varzea areas (subject to periodic flooding). The author also pointed out that there is considerable variation in the natural populations regarding the characteristics of trunk circumference, period of seed fall and size, bark type, crown form, and tree density per hectare.

On the other hand, Paiva (1981), observing the native rubber tree stands in the state of Mato Grosso, pointed out the existence of a lower variability among the populations on terra firme, in comparison with those in the varzea, as to the characteristics of form, size and colour of seeds. He also

suggested the hypothesis that the rubber tree groves in the varzea should have more genetic variability due to the dispersion mechanism of the rivers, which favours more dispersion (genetic flow) and a higher concentration of different types per area occupied.

For the two rubber tree populations studied, Nei's estimates for the normalized genetic identity rate showed a value of 0.997 and 0.003 for the genetic difference value, indicating that the two populations are quite closely related genetically.

The genetic diversity between the two populations estimated by the D_{ST} (Nei 1973), F_{ST} (Wright 1978) and \hat{e} (Weir 1990) rates for the average of the four loci in the two populations was also very low (Table 4).

As can be observed, the estimates evaluating the genetic differences between populations, utilizing different methodologies showed low and similar values, leading to the conclusion that the two populations studied are genetically alike. This was to be expected, to a certain point, considering the distance between the groves and the fact that both stands are on terra firme, as compared to rubber tree groves in varzea, they are more phenotypically similar. These results also show that there did not exist genetic flow between the two groups of plants, since despite the apparent discontinuity between the two groups, the differentiation process was still shown to be incipient.

Tropical ecosystems, undoubtedly present the greatest diversity of species and level of complexity of inter-relationships between organisms. The

TABLE 4. Estimates of the genetic distance for the 17 alleles in 4 loci of the two natural populations of rubber trees.¹

LOCI	NEI (1973)	WRIGHT (1978)	WEIR (1990)
	D_{ST}	F_{ST}	\hat{e}_2
LAP-1	0,0013	0,0040	0,0000
LAP-2	0,0005	0,0030	-0,0001
SKDH	0,0014	0,0020	-0,0008
MDH-1	0,0014	0,0180	0,0531
MEAN	0,0001	0,0067	0,0025

¹Adapted from Paiva (1992).

diversity results from the sum of several interacting factors in the ecosystem. As a consequence the individuals of a specie remain somewhat isolated among other individuals of other species. This has implications for forest dynamics, in terms of plant-animal interactions, which are interdependent and well co-adapted, integrating and stabilizing the ecosystem (Pianka 1983).

Based upon these arguments, one may infer that each specie of the tropical forest has developed its own adaptation strategies, seeking to live in harmony and dynamic equilibrium with the heterogeneity of the ecosystem. Furthermore, one may infer that the strategies utilized by one specie may not serve as a reference for other species.

Thus it is reasonable to assume that the rate of natural cross-fertilization estimated for the rubber tree, although low according to standards of species held to be allogamous, as well as the degree of endogamy, may be considered as biological strategies developed by the species in order to maintain equilibrium in the tropical ecosystem. It is well known that the rubber tree cultivated plantations in a tropical environment is decimated by the pathogen (*Microcyclus ulei*) but nonetheless, lives in harmony with this fungus in natural populations, which lends credence to the thesis presented.

CONCLUSIONS

- The degree of variability estimated in the two populations shows high levels of genetic

variation, the greater part being found within the population;

- Parameters which measure the genetic difference between the two populations indicate that they are similar and that the collection of germ plasm for this specie should be directed to one population, with the highest number of individuals;
- The low estimates of cross fertilization rates for the rubber tree in natural populations indicates a tendency in this specie to belong to the intermediate plant group, in terms of its reproductive system.

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