

## DIVERSITY, INBREEDING AND INBREEDING DEPRESSION IN RUBBER TREE (*Hevea* spp.)

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Based on pedigree information, diversity, inbreeding level and inbreeding depression were estimated among clones released to rubber producers from 1950 to 2000, by the Rubber Research Institute of Malaysia (RRIM), the Cacao Research Center (CEPEC) and the Agronomic Institute of Campinas (IAC), in Brazil. Fifty founding or primary clones were used to produce all varieties of the three breeding programs, however some of them were overused, resulting in low diversity in plantations ( $N_e < 22$ ). The clones Tjir 1, PR 107 and PB 86, together, contributed on average 44% of all alleles of the 42 varieties released by RRIM and 38% of those 22 released by IAC. PB 86, F 4542 and Tjir 1 contributed 42% of all alleles of the 35 clonal varieties recommended by CEPEC. Using unpublished and published information by several authors, it was shown in this paper that inbreeding depression occurs in rubber tree. In the populations studied, yield reduced 10.1 g/tree/tapping in adult trees for each 0.1 units of increase in the inbreeding coefficient (F). Bark thickness reduced 0.8 mm per each 0.1 F, trunk girth 2.3 to 11.4 cm in mature trees and 2.4 cm in young trees. South American Leaf Blight severity increased 0.03 points, per each 0.1 F, in a scale of severity ranging from 1 (plants free of infection) to 4 (highest level of SALB infection). Based on these results, increasing diversity in the programs studied appears to be mandatory in order to reduce future depression by endogamy and the risks with disease epidemics.

**Key words:** breeding, genetic diversity, leaf blight, clone, yield

**Diversidade, endogamia e depressão por endogamia em clones de seringueira (*Hevea* spp.).** Baseado em informações de ancestralidade, a diversidade, o nível e a depressão por endogamia foram estimadas entre clones recomendados para os produtores entre 1950 e 2000, pelo Instituto de Pesquisa da Borracha da Malásia (RRIM), Centro de Pesquisas do Cacau (CEPEC) e Instituto Agrônomo de Campinas (IAC), no Brasil. Cinquenta clones primários ou fundadores foram envolvidos no desenvolvimento de todas as variedades destes programas de melhoramento, entretanto alguns deles foram excessivamente usados, resultando em uma baixa diversidade nos plantios comerciais ( $N_e < 22$ ). Os clones Tjir 1, PR 107 e PB 86, juntos, contribuíram em média com 44% de todos os alelos das 42 variedades recomendadas pelo RRIM e 38% daquelas 22 recomendadas pelo IAC. O PB 86, F 4542 e Tjir 1 contribuíram com 42% dos alelos das 35 variedades clonais recomendadas pelo CEPEC. Usando informações não publicadas e publicadas por vários autores, foi mostrado neste artigo que depressão por endogamia ocorre em seringueira. Nas populações estudadas, constatou-se uma redução na produção de 10,1 g/árvore/corte em árvores adultas para cada 0.1 unidade de incremento no coeficiente de endogamia (F). Espessura da casca foi reduzida de 0.8 mm para cada 0.1 F, perímetro do tronco de 2.3 a 11.4 cm em árvores adultas e 2.4 cm em plantas jovens. A severidade do mal-das-folhas aumentou 0.03 pontos, para cada 0.1F, considerando-se uma escala de notas variando de 1 (plantas livres de infecção) a 4 (nível mais alto de infecção da doença). Com base nestes resultados, o aumento da diversidade nos programas estudados torna-se obrigatório, com o objetivo de reduzir futura depressão por endogamia e os riscos de epidemias com doenças.

**Palavras-chave:** Melhoramento, diversidade genética, mal-das-folhas, clones, produção

## Introduction

The genus *Hevea* is the most important source of natural rubber. The genus has 11 species, but only five are used in breeding programs, namely: *Hevea brasiliensis* (Willd. ex A.D. de Juss.) Muell.-Arg., *Hevea benthamiana* Muell.-Arg., *Hevea pauciflora* (Spruce ex Benth.) Muell.-Arg., *Hevea comaragoana* Pires and *Hevea comporum* Ducke (Gonçalves et al., 1997). Among them, *H. brasiliensis* is the most frequently used for rubber production. Despite the wide variability within and across species in the genus, rubber tree breeding programs have often been cited as based on a narrow genetic base (Tan, 1987). The small diversity available to the early breeding programs, assortative mating, directional selection for high yield and the extensive use of clones as varieties are the main reasons for the current low diversity (Tan, 1987; Yee, 1980).

In Asia, the first plantations and breeding programs were based on a few plants (around 2,000 to 3,000) introduced in 1876, from a small portion of the diversity center, the Tapajós basin area, in the Brazilian Amazon (Imle, 1978). Malaysia, the largest natural rubber producer and with one of the most active breeding programs worldwide, received only 22 of those seedlings (Imle, 1978). Moreover, only a small portion of those was substantially used in breeding (Wycherley, 1969). A similar story occurred in Latin America. In Brazil, the first selections were made in plantations of unimproved seedlings also from the Tapajós basin (Gonçalves et al., 1997). Only later, seeds from other Amazon regions were used, particularly in crosses with clones introduced from Malaysia (Gonçalves et al., 1997).

Inbreeding depression in rubber tree was firstly reported by Sharp (1940). Later, Sharp (1951) observed that the vigor of crosses involving unrelated clones was larger than that involving half-sibs, and this, larger than that of full-sibs. The same trend was observed by Ross and Brookson (1966). Gilbert et al. (1973), re-analyzing the data presented by Ross and Brookson (1966), confirmed the presence of inbreeding depression. In 1976, Tan and Subramanian (1976b) showed that selfed progenies, in the nursery, were inferior in vigor and yield than outcrossed families.

In this study diversity and inbreeding of clonal varieties released for planting from 1950s to 2000, by three large rubber tree breeding programs, in Malaysia and Brazil, and the level of inbreeding depression for several agronomic traits were estimated.

## Materials and methods

The effective population size, the genetic contribution of founding or primary clones, the inbreeding coefficient, the inbreeding depression and the coancestry coefficient were estimated among clones recommended for planting at different periods, by the Rubber Research Institute of Malaysia (RRIM), by the Agronomic Institute of Campinas (IAC), and by the Cacao Research Center (CEPEC), in Brazil. The pedigrees of the rubber tree clones were obtained from literature (Bahia et al., 1985; Gilbert et al., 1973; Gonçalves et al., 1993; Gonçalves, 1998; Khoo & Ong, 1989; Ong et al., 1995; RRIM, 1955; 1957; 1959; 1961; 1963; 1965; 1967; 1969; 1971a; 1971b; 1977a; 1977b; 1980; 1983; 1986; 1989) and breeding program records.

**Coancestry coefficient (f):** The coancestry coefficient between two clones is the degree of relationship by descent between them, and is equal to the inbreeding coefficient of the progeny of those clones if they were crossed (Falconer, 1989). Coancestry coefficients are useful for planning crosses with minimum (or maximum) inbreeding, and also for evaluating genetic diversity. The coancestry coefficients were computed as described by Falconer (1989), using the INBREED procedure from SAS® (SAS Institute Inc., 1996), for all possible pairs of clones within and across the three breeding programs.

**Effective population size (Ne):** The status effective population size is the number of unrelated and non-inbred individuals in an *ideal* panmitic population that would produce progeny with the same average coefficient of inbreeding as the progeny of the individuals of the breeding population under consideration mated at random (Falconer, 1989). The effective population size is useful for evaluating the genetic diversity in populations. This number was computed as (Lindgren et al., 1996):

$$N_e = 0.5/\Phi$$

where  $N_e$  is the effective population size and  $F$  is the average coancestry of the population, computed from the matrix of coancestries of all possible clone combinations in the population considered. In order to compare the effective population sizes in different breeding programs, at the same basis,  $N_e$  was divided by  $N$ , the actual number of varieties.

**Genetic contribution of founding clones (GC):**

Estimates of the genetic contribution (GC) of founding clones for the genetic make-up of varieties released is important in assessing diversity and alerting about the overuse of certain clones in detriment of others. These estimates were obtained as described by Sjuln and Dale (1987). Briefly, assuming that each parent of a given clone contributes with  $\frac{1}{2}$  of the alleles of that clone, the grandparents  $\frac{1}{4}$ , and so on, the percent of alleles contributed by individuals in the pedigree of a clonal variety can be computed as:

$$GC_j = \left[ \sum_{i=1}^c GC_{j(i)} \right] / [C * 100]$$

where  $GC_j$  is the genetic contribution of the  $j^{\text{th}}$  founding clone to all clonal varieties released in the period and  $C$  the number of varieties released in that period. The genetic contribution and the frequency of participation in pedigrees were computed for each founding clone and period.

**Inbreeding coefficient (F):** This coefficient is the probability of two alleles at a given locus from an individual being identical by descent, i.e., the probability they are replicates of the same allele in previous generations (Wright, 1922). It was computed as described in Falconer (1989), by using the INBREED SAS® procedure (SAS Institute Inc., 1996). Parents of unknown origin were assumed non-inbred and unrelated, considering that the flowers of *Hevea* spp. are unisexual and the trees are monoecious and largely cross-pollinated. However, it is likely that some founding clones are already inbred, as pointed by Imle (1978), resulting here in underestimated inbreeding coefficients.

**Inbreeding depression:** It was computed for several traits, using unpublished and published data by several authors (Alika & Onopkise, 1982; Gilbert et al., 1973; Ross & Brookson, 1966; Sharp, 1951; Tan, 1979; Tan & Subramaniam, 1976a). In order to standardize the data, the yield units were transformed to grams/tree/tapping and bark thickness to millimeter. Also, instead of working with trunk diameter, they were transformed to girth and expressed in centimeters.

The first dataset used was that from CEPEC's program. A total of 901 seedlings, from 121 full-sib families, in different small-scale tests, were considered in this study. Some families had their yield measured

by precocious yield tests, like the MTP (Minitest) and the HMMM (Hamaker-Morris-Mann) tests (Morris and Mann, 1932; Mendes, 1971). Bark thickness was measured at 3 years of age, trunk girth at 1 and 3 years, pollination and germination successes and South American Leaf Blight (SALB) severity, at 3 to 4 years. SALB severity was assessed by a scale of ratings ranging from 1 (plants free of infection) to 4 (highest level of SALB infection).

The second dataset was that published initially by Sharp (1951) and re-analyzed by Ross and Brookson (1966) and Gilbert et al. (1973). In 1938, a full diallel involving six parents was planned. However, only 14 families were obtained and analyzed by Sharp (1951). Some families with low number of individuals were discarded in Ross and Brookson's (1966) analyses. Here, the same data used by Ross and Brookson (1966) were used.

The third dataset was that from Tan (1979), and constitutes a single pair mating design. The variables measured by the author and used here to estimate inbreeding depression were: yield, girth at opening, girth after 5 years of tapping, girth increment over five years, virgin bark thickness, and thickness of renewed bark.

The fourth dataset was that from Alika and Onopkise (1982). The data are from a single pair mating design formed by selecting 10 crosses among those from phase I of the RRIM's program and include the variables: yield, girth at 4 years of tapping, panel B virgin bark thickness, and renewed bark thickness after 4 years of tapping.

The fifth dataset was that from Tan and Subramanian (1976a), collected from a full diallel involving five parents with high general combining ability. The variables measured were yield, diameter, bark thickness, latex vessel number, latex vessel size, sieve tube size and plugging index.

Inbreeding depression was computed by fitting a linear regression of the family average phenotypic value, for each trait, on the family inbreeding coefficient, such that:

$$P = \beta_0 + \beta_1(F)$$

where  $P$  is the phenotypic family averages,  $F$  is the inbreeding coefficient of the families,  $b_0$  is the intercept and  $b_1$  the slope of the regression.  $b_1$  is an estimate of

how much the family averages will be changed per each unit of inbreeding coefficient. However, as the inbreeding coefficient ranges from 0 to 1, inbreeding depression is better expressed in 0.1 units of that coefficient.

As most of the crosses used here for studying inbreeding depression were not planned for this purpose, different breeding values occur among these crosses. In this study, it was assumed no relationship between inbreeding coefficient and genetic value of the crosses. Therefore, it is not expected (randomly or not) that crosses with a high inbreeding coefficient will consistently have low genetic values (overestimating or causing a false estimate of inbreeding depression) or consistently high values (underestimating the depression). However, for groups of crosses for which both phenotypic and genetic values (general combining abilities) were available, an estimate of the inbreeding depression free of the effect of genetic value was obtained. This was done by adding the term associated to the genetic value ( $G$ ) in model 5, such that:

$$P = \beta_0 + \beta_1(F) + \beta_2(G)$$

Where  $P$ ,  $b_0$  and  $F$  are the same as defined for model 4,  $G$  is the genetic value of the cross, obtained by adding the two general combining abilities of the parents involved in the cross and  $b_2$  is the regression coefficient associated to the genetic value.  $b_1$  in model 5 estimates the linear effect of the inbreeding coefficient ( $F$ ) on the phenotypic value ( $P$ ), after discounting the linear effects of the genetic value of the cross on both the inbreeding coefficient ( $F$ ) and the phenotypic value ( $P$ ).

## Results

### Coancestry coefficient

Average coancestry coefficients are presented in Table 1 for each breeding program. The largest

coancestry occurred among varieties released by RRIM ( $f=0.042$ ) and the smallest among those released by IAC ( $f = 0.023$ ). In addition, clones released by RRIM and CEPEC are quite unrelated each other; while clones released by IAC are related to those released by both RRIM and CEPEC. About 63% (544/861), 82% and 76% of the pairs of clones recommended by RRIM, IAC and CEPEC, respectively, are unrelated ( $f=0$ ); while only 6%, 3%, and 5% presented coancestry larger than 0.24 (Table 1).

### Effective population size

The effective population size estimated for clones recommended by the three programs ranged from 11.9 in RRIM's program to 21.7 in IAC's program (Table 1). The relative effective population size ( $Ne/N$  ratio) was minimum for RRIM's program (0.28) and maximum for IAC's program (0.99).

### Genetic contribution of founding clones

All varieties recommended from 1950s to 2000, by the three programs, can be tracked back to only 50 founding clones (Table 2). Among these, 24 contributed alleles for at least two breeding programs. Tjir 1 and PB 86 contributed the largest proportion of alleles for the clones released by the three programs.

The development of the 42 varieties released from 1955 to 1997, RRIM involved only 27 founding clones (Table 2). Among them, Tjir 1, PR 107 and PB 86 contributed 44% of the alleles of those varieties and participated in pedigrees of up to 67, 50 and 25%, respectively, in some stages of the program (Table 3). Until 1991, no more than nine founding clones contributed alleles in the formation of RRIM varieties. GT 127, GT 711 and Harbel 1 participated only in the first stages of the program (1956-1967), while B 38, B 45, F 1619, B 110, CD 1130, F 170, F 315, only in late stages (1973-2000).

Table 1. Average coancestry coefficients among clonal varieties, number of pairs of varieties for each level of coancestry, actual number (N), effective population number (Ne) and the relative effective number (Ne/N), for the breeding programs in the Rubber Research Institute of Malaysia (RRIM), the Agronomic Institute of Campinas (IAC), and the Cacao Research Center (CEPEC)

Program	Coancestry			Number of Pairs of Varieties				N	Ne	Ne/N
	RRIM	IAC	CEPEC	0	0.01-0.12	0.13-0.24	> 0.24			
RRIM	0.042	0.023	0.014	544	137	132	48	42	11.9	0.28
IAC		0.023	0.026	190	14	19	8	22	21.7	0.99
CEPEC			0.034	451	26	87	31	35	14.7	0.42

Table 2. Genetic contribution of founding clones for the clonal varieties released by the Rubber Research Institute of Malaysia (RRIM), the Agronomic Institute of Campinas (IAC) and the Cacao Research Center (CEPEC), respectively, from 1955 to 1997, 1956 to 1998 and 1956 to 2000

Founding <sup>a</sup>	RRIM											IAC					CEPEC				
	55 to 60	61 to 62	63 to 64	65 to 66	67 to 72	73 to 76	77 to 82	83 to 91	92 to 94	95 to 97	55 to 97	56 to 70	71 to 80	80 to 98	56 to 98	56 to 67	68 to 72	73 to 84	85 to 84	56 to 0	
Tjir 1	17	20	25	10	13	25	30	29	13	12	18	18	7	7	10	9	14	10	6	0	11
PR 107	17	20	25	20	25	33	20	17	6	10	17	17	14	14	3	8	-	-	-	-	-
PB 86	17	20	0	0	13	8	10	8	6	7	9	9	29	29	15	21	14	20	17	33	17
GT 1	0	0	0	0	25	17	20	17	6	2	9	9	0	0	6	3	-	-	-	-	-
PB 24	0	0	13	10	13	8	5	4	9	13	8	8	0	0	4	2	-	-	-	-	-
PB 56	0	0	13	10	13	8	5	4	9	13	8	8	-	-	-	-	-	-	-	-	-
PB 49	0	0	0	20	0	0	0	4	9	6	4	4	0	0	1	1	2	0	6	0	3
PB 6-9	0	0	0	0	0	0	10	8	2	4	3	3	-	-	-	-	-	-	-	-	-
Gl 1	17	20	0	0	0	0	0	0	0	0	3	3	-	-	-	-	-	-	-	-	-
Pil A 44	17	10	13	10	0	0	0	0	2	2	4	4	0	0	1	1	2	0	0	0	1
PB 280	0	0	0	0	0	0	0	0	4	4	2	2	-	-	-	-	-	-	-	-	-
PB 28-59	0	0	0	0	0	0	0	0	4	6	2	2	-	-	-	-	-	-	-	-	-
Lun N	8	0	0	0	0	0	0	0	2	2	2	2	0	0	1	1	-	-	-	-	-
Pil B 16	8	10	13	10	0	0	0	0	0	0	2	2	-	-	-	-	2	0	0	0	1
Pil B 84	0	0	0	10	0	0	0	0	6	4	2	2	-	-	-	-	-	-	-	-	-
PM 10	0	0	0	0	0	0	0	0	4	4	2	2	-	-	-	-	-	-	-	-	-
AVROS 49	0	0	0	0	0	0	0	0	3	0	1	1	7	7	1	4	2	0	0	0	1
PB 25	0	0	0	0	0	0	0	0	2	1	1	1	0	0	1	1	-	-	-	-	-
RRIM 71	0	0	0	0	0	0	0	8	2	2	2	2	-	-	-	-	-	-	-	-	-
PB 32-36	0	0	0	0	0	0	0	0	2	2	1	1	0	0	6	3	-	-	-	-	-
F 351	0	0	0	0	0	0	0	0	1	2	1	1	7	7	1	4	2	0	0	0	1
RRIC 52	0	0	0	0	0	0	0	0	2	2	1	1	-	-	-	-	-	-	-	-	-
PB 202	0	0	0	0	0	0	0	0	0	2	0	0	-	-	-	-	-	-	-	-	-
AVROS 374	0	0	0	0	0	0	0	0	1	0	0	0	7	7	0	3	5	0	0	0	3
LCB 1320	0	0	0	0	0	0	0	0	2	0	0	0	-	-	-	-	-	-	-	-	-
AVROS 214	0	0	0	0	0	0	0	0	1	0	0	0	7	7	3	5	2	0	0	0	1
RRIC 7	0	0	0	0	0	0	0	0	2	0	0	0	-	-	-	-	-	-	-	-	-
F 4542	-	-	-	-	-	-	-	-	-	-	-	-	0	0	6	4	17	33	1	0	14
AVROS 363	-	-	-	-	-	-	-	-	-	-	-	-	0	0	3	2	7	20	0	0	7
AVROS 256	-	-	-	-	-	-	-	-	-	-	-	-	7	7	0	3	7	0	0	0	4
AVROS 26	-	-	-	-	-	-	-	-	-	-	-	-	0	0	4	2	0	4	13	8	4
AVROS 317	-	-	-	-	-	-	-	-	-	-	-	-	7	7	3	5	-	-	-	-	-
AVROS 368	-	-	-	-	-	-	-	-	-	-	-	-	0	0	3	2	2	0	0	0	1
B 38	-	-	-	-	-	-	-	-	-	-	-	-	0	0	3	2	0	0	6	17	3
B 45	-	-	-	-	-	-	-	-	-	-	-	-	0	0	3	2	0	0	11	0	3
F 1619	-	-	-	-	-	-	-	-	-	-	-	-	0	0	3	2	0	0	6	17	3
FA 1717	-	-	-	-	-	-	-	-	-	-	-	-	7	7	3	5	0	10	0	0	1
PB 186	-	-	-	-	-	-	-	-	-	-	-	-	0	0	3	2	-	-	-	-	-
PB 32-35	-	-	-	-	-	-	-	-	-	-	-	-	0	0	3	2	-	-	-	-	-
PB 56	-	-	-	-	-	-	-	-	-	-	-	-	0	0	4	2	-	-	-	-	-
B 110	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	6	17	3
CD 1130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	6	0	1
F 170	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	6	0	1
F 315	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	6	0	1
F 409	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	0	0	3
GT 127	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	0	0	3
GT 711	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	0	0	3
Harbel 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	0	0	3
Unknown 1	-	-	-	-	-	-	-	-	-	-	-	-	0	0	4	2	0	4	13	8	4
Unknown 2	-	-	-	-	-	-	-	-	-	-	-	-	0	0	3	2	-	-	-	-	-

<sup>a</sup> Unknown 1 is the unknown parent of AVROS 183 and Unknown 2 that one of PB 253.



Table 3. Frequency of participation (%) of founding clones in pedigrees of clonal varieties released by the Rubber Research Institute of Malaysia (RRIM), Agronomic Institute of Campinas (IAC), and Cacao Research Center (CEPEC), respectively, from 1955 to 1997, 1956 to 1998 and 1956 to 2000

Founding <sup>a</sup>	RRIM											IAC				CEPEC				
	55 to 60	61 to 62	63 to 64	65 to 66	67 to 72	73 to 76	77 to 82	83 to 91	92 to 94	95 to 97	97 to 97	56 to 70	71 to 80	80 to 98	56 to 98	56 to 67	68 to 72	73 to 84	85 to 0	56 to 0
Tjir 1	17	20	25	20	25	50	60	67	40	42	40	14	14	22	19	24	20	11	0	18
PR 107	17	20	25	20	25	50	40	33	12	21	25	14	14	6	9	-	-	-	-	-
PB 86	17	20	0	0	25	17	20	17	16	21	17	43	43	33	38	24	40	33	67	32
GT 1	0	0	0	0	25	17	20	17	12	4	11	0	0	6	3	-	-	-	-	-
PB 24	0	0	25	20	25	17	20	17	36	54	26	0	0	17	9	-	-	-	-	-
PB 56	0	0	25	20	25	17	20	17	36	54	26	-	-	-	-	-	-	-	-	-
PB 49	0	0	0	40	0	0	0	17	28	21	13	0	0	6	3	5	0	11	0	5
PB 6-9	0	0	0	0	0	0	20	17	4	13	7	-	-	-	-	-	-	-	-	-
Gl 1	17	20	0	0	0	0	0	0	0	0	3	-	-	-	-	-	-	-	-	-
Pil A 44	33	20	25	20	0	0	0	0	12	13	11	0	0	6	3	5	0	0	0	3
PB 280	0	0	0	0	0	0	0	0	4	4	2	-	-	-	-	-	-	-	-	-
PB 28-59	0	0	0	0	0	0	0	0	4	8	2	-	-	-	-	-	-	-	-	-
Lun N	17	0	0	0	0	0	0	0	12	13	7	0	0	6	3	-	-	-	-	-
Pil B 16	17	20	25	20	0	0	0	0	0	0	5	-	-	-	-	5	0	0	0	3
Pil B 84	0	0	0	20	0	0	0	0	24	21	9	-	-	-	-	-	-	-	-	-
PM 10	0	0	0	0	0	0	0	0	4	4	2	-	-	-	-	-	-	-	-	-
AVROS 49	0	0	0	0	0	0	0	0	8	0	2	14	14	6	9	5	0	0	0	3
PB 25	0	0	0	0	0	0	0	0	8	4	2	0	0	6	3	-	-	-	-	-
RRIM 71	0	0	0	0	0	0	0	17	4	4	4	-	-	-	-	-	-	-	-	-
PB 32-36	0	0	0	0	0	0	0	0	4	4	2	0	0	11	6	-	-	-	-	-
F 351	0	0	0	0	0	0	0	0	4	4	2	14	14	6	9	5	0	0	0	3
RRIC 52	0	0	0	0	0	0	0	0	4	4	2	-	-	-	-	-	-	-	-	-
PB 202	0	0	0	0	0	0	0	0	0	4	1	-	-	-	-	-	-	-	-	-
AVROS 374	0	0	0	0	0	0	0	0	4	0	1	14	14	0	6	10	0	0	0	5
LCB 1320	0	0	0	0	0	0	0	0	4	0	1	-	-	-	-	-	-	-	-	-
AVROS 214	0	0	0	0	0	0	0	0	4	0	1	14	14	6	9	5	0	0	0	3
RRIC 7	0	0	0	0	0	0	0	0	4	0	1	-	-	-	-	-	-	-	-	-
F 4542	-	-	-	-	-	-	-	-	-	-	-	0	0	22	13	33	80	11	0	32
AVROS 363	-	-	-	-	-	-	-	-	-	-	-	0	0	11	6	14	40	0	0	13
AVROS 256	-	-	-	-	-	-	-	-	-	-	-	14	14	0	6	14	0	0	0	8
AVROS 26	-	-	-	-	-	-	-	-	-	-	-	0	0	17	9	0	20	56	33	18
AVROS 317	-	-	-	-	-	-	-	-	-	-	-	14	14	6	9	-	-	-	-	-
AVROS 368	-	-	-	-	-	-	-	-	-	-	-	0	0	6	3	5	0	0	0	3
B 38	-	-	-	-	-	-	-	-	-	-	-	0	0	6	3	0	0	11	33	5
B 45	-	-	-	-	-	-	-	-	-	-	-	0	0	6	3	0	0	22	0	5
F 1619	-	-	-	-	-	-	-	-	-	-	-	0	0	6	3	0	0	11	33	5
FA 1717	-	-	-	-	-	-	-	-	-	-	-	14	14	6	9	0	20	0	0	3
PB 186	-	-	-	-	-	-	-	-	-	-	-	0	0	6	3	-	-	-	-	-
PB 32-35	-	-	-	-	-	-	-	-	-	-	-	0	0	6	3	-	-	-	-	-
PB 56	-	-	-	-	-	-	-	-	-	-	-	0	0	17	9	-	-	-	-	-
B 110	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	11	33	5
CD 1130	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	11	0	3
F 170	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	11	0	3
F 315	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0	11	0	3
F 409	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10	0	0	0	5
GT 127	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	0	0	3
GT 711	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	0	0	3
Harbel 1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	0	0	0	3
Unknown 1	-	-	-	-	-	-	-	-	-	-	-	0	0	17	9	0	20	56	33	18
Unknown 2	-	-	-	-	-	-	-	-	-	-	-	0	0	6	3	-	-	-	-	-

<sup>a</sup>Unknown 1 is the unknown parent of AVROS 183 and Unknown 2 that one of PB 253.

In the development of the 22 clones, IAC used 29 founding clones (Table 2). Among them, PB 86, Tjir 1 and PR 107 contributed 38% of all alleles. Nevertheless, the participation of PB 86 and PR 107 decreased from 1956 to 1998, while that of Tjir 1 increased.

### Inbreeding coefficient

The inbreeding coefficient for all varieties developed by RRIM, CEPEC, and IAC estimated in this study was zero (results not shown).

### Inbreeding depression

A negative slope of the linear regression of phenotypic values on inbreeding coefficients was observed for most traits studied here, indicating that

increasing those coefficients, the phenotypic values decrease, resulting in inbreeding depression (Table 4). Twelve out of 25 negative coefficients were significant and had coefficients of determination higher than 0.19 (average 0.56). For all cases in which the slope was positive, excepting for SALB severity, the regressions had low determination coefficients ( $R^2 \leq 0.07$ ), and then the regressions provide little information about the relationship between the phenotypic values and the inbreeding coefficient.

As a result of inbreeding depression, for each 0.1 units increase in the inbreeding coefficient (F), yield reduced up to 4.80 and 6.61 g/tree/tapping; trunk girth up to 0.44 and 11.40 cm; bark thickness up to 0.35

Table 4 Regression coefficients ( $b_0$ = intercept,  $b_1$ =slope associated to inbreeding), coefficient of determination of the regression on the inbreeding coefficient of progenies of rubber tree, number of pairs of points (N), for several traits, using published data from several authors and generated by CEPEC's breeding program

Trait	N	Approx. Age (years)	Unit	$\beta_0$	$\beta_1$	$R^2$	Authors
Yield (MTP)	6	1	g/tree/tap	10.2**	-48.0*	0.67	CEPEC Unpublished
Yield (HMM)	24	2	g/tree/tap	6.7**	-7.2*	0.54	Tan & Subramanian (1976a)
Yield (HMM)	3	3	g/tree/tap	2.9 <sup>ns</sup>	-5.3 <sup>ns</sup>	0.22	CEPEC Unpublished
Yield	8	6-9	g/tree/tap	37.0**	-28.7 <sup>ns</sup>	0.17	Ross & Brookson (1966)
Yield	8	6-21	g/tree/tap	41.6**	-66.1**	0.74	Ross & Brookson (1966)
Yield	10	6-10	g/tree/tap	26.2**	13.1 <sup>ns</sup>	0.04	Alika & Onopkise (1982)
Yield	21	6/10-11/15	g/tree/tap	39.9**	-24.9 <sup>ns</sup>	0.07	Tan (1979)
Trunk Girth	7	1	cm	6.1**	-9.6 <sup>ns</sup>	0.41	CEPEC Unpublished
Trunk Girth	24	2	cm	18.2**	-4.4 <sup>+</sup>	0.14	Tan & Subramanian (1976a)
Trunk Girth	3	3	cm	20.3*	-24.4 <sup>ns</sup>	0.97	CEPEC Unpublished
Trunk Girth	21	6/10	cm	57.2**	-20.5 <sup>ns</sup>	0.07	Tan (1979)
Trunk Girth	8	9	cm	72.7**	-57.5**	0.77	Ross & Brookson (1966)
Trunk Girth	10	10	cm	83.8**	9.9 <sup>ns</sup>	0.07	Alika & Onopkise (1982)
Trunk Girth	21	11/15	cm	79.0**	-30.5*	0.27	Tan (1979)
Trunk Girth	8	15	cm	96.8**	-114.0**	0.80	Ross & Brookson (1966)
Trunk Girth Increment	8	9-15	cm	24.5**	-59.1**	0.73	Ross & Brookson (1966)
Trunk Girth increment	21	6/10-11/15	cm	19.4**	-9.8 <sup>ns</sup>	0.02	Tan (1979)
Bark Thickness	24	2	mm	3.5**	-0.8*	0.20	Tan & Subramanian (1976a)
Bark Thickness	4	3	mm	2.7*	-5.8 <sup>ns</sup>	0.49	CEPEC Unpublished
Virgin Bark Thickness	10	10	mm	14.4**	-8.6*	0.54	Alika & Onopkise (1982)
Virgin Bark Thickness	21	11/15	mm	9.6**	-3.5 <sup>+</sup>	0.17	Tan (1979)
Renewed Bark Thickness	10	10	mm	10.7**	-5.5 <sup>ns</sup>	0.28	Alika & Onopkise (1982)
Renewed Bark Thickness	21	11/15	mm	9.1**	-3.0 <sup>+</sup>	0.14	Tan (1979)
Sieve Tube Size	24	2	$\mu$	15.2**	-1.2*	0.26	Tan & Subramanian (1976a)
Plugging Index	24	2	-	11.6**	0.9 <sup>ns</sup>	0.01	Tan & Subramanian (1976a)
Latex Vessel Number	24	2	-	6.8**	-1.0 <sup>ns</sup>	0.08	Tan & Subramanian (1976a)
Latex Vessel Size	24	2	$\mu$	11.0**	-0.7*	0.19	Tan & Subramanian (1976a)
Germination Success	8	0-1	%	59.6**	5.8 <sup>ns</sup>	0.01	CEPEC Unpublished
Pollination Success	7	-	%	3.3**	-2.2 <sup>ns</sup>	0.08	CEPEC Unpublished
SALB Severity	6	3-4	Scale 1-4	2.3**	3.3 <sup>ns</sup>	0.34	CEPEC Unpublished

+ Significant by t test ( $\alpha = 0.10$ )  
ns Non significant

\*Significant by t test ( $\alpha = 0.05$ )

\*\*Significant by t test ( $\alpha = 0.01$ )

and 0.86 mm, in young and adult trees, respectively (Table 4). Although the slope was not statistically significant for SALB severity, it was observed an increase of 0.03 points in a scale of severity ranging from 1 to 4, per each 0.1 units increase in F. This indicates that increasing F, SALB resistance decreases.

Regression analyses including both the inbreeding coefficient and the genetic value of the cross, obtained from the GCA of the parents, showed that the regression coefficients associated to genetic values were always positive for the data analyzed (Table 5), indicating that, as the genetic values of the parents increase, the phenotype measurements also increase.

In general, even adjusting the genetic value of the crosses, the regression coefficients associated to the effect of inbreeding ( $b_1$ ) were negative. Therefore, even after the adjustment, when endogamy increases, the value of the traits decreases, resulting in endogamy depression.

## Discussion

The repeated use of superior genotypes in rubber tree breeding programs has reduced the diversity, reducing the chances of genetic gain in advanced generations and increasing the chances of epidemics and inbreeding depression. Moreover, the exchange of genotypes between programs has resulted in common use of some clones as parents, increasing relatedness across programs.

Although the clonal varieties released by RRIM, IAC and CEPEC are still not inbred, coancestry among them exists, as a result of the frequent use of some specific clones. In CEPEC's program, only 27 founding clones were used and preference was given for the high yielder clones Tjir 1 and PB 86 and for one with some resistance to SALB, F 4542. In RRIM's and IAC's programs, for which SALB is not important, less than 29 founding clones were used and preference was given to the high yielder clones Tjir 1, PB 86, and PR 107, among others. Tjir 1, PR 107 and PB 86 contributed on average with 38 and 44% of the alleles of all varieties recommended for planting by IAC and by RRIM, respectively; and Tjir 1, PB 86 and F 4542 42% of the alleles of the varieties recommended by CEPEC. So, most of the trees in plantations in the two countries (Brazil and Malaysia), even being from varieties with different names, carry a large portion of similar alleles, coming from a few founding clones. This low diversity in plantations put them under risk of diseases and insects attack and non-adaptation to changes in climate. Moreover, the clones Tjir 1, PB 86 and PR 107, widely used in the programs of RRIM and IAC, present no resistance to diseases such as South American Leaf Blight.

The low diversity among the varieties recommended by the three breeding programs can also be shown by the low effective population sizes observed in this study

Table 5 Regression coefficients ( $b_0$ =intercept,  $b_1$ =slope associated to inbreeding,  $b_2$ =slope associated to genetic value), coefficient of determination of the regression on the inbreeding coefficient and genetic value of progenies of rubber tree, for several traits, using published data from several authors and generated by CEPEC's breeding program.

Trait	Approx Age (years)	Unit	$\beta_0$	$\beta_1$	$\beta_2$	R <sup>2</sup>	Authors
Bark Thickness	2	mm	3.5**	-0.5**	1.0**	0.83	Tan & Subramanian (1976a)
Yield (HMM)	2	g/tree/tap	6.6**	-7.0**	0.3 <sup>ns</sup>	0.58	Tan & Subramanian (1976a)
Yield	6-9	g/tree/tap	10.2 <sup>ns</sup>	-17.0 <sup>ns</sup>	0.8 <sup>ns</sup>	0.31	Ross & Brookson(1966)
Yield	6-21	g/tree/tap	22.5 <sup>ns</sup>	-43.0 <sup>ns</sup>	0.5 <sup>ns</sup>	0.80	Ross & Brookson(1966)
Trunk Girth	2	cm	18.0**	-2.4 <sup>ns</sup>	0.6**	0.51	Tan & Subramanian (1976a)
Trunk Girth	9	cm	-174.7 <sup>ns</sup>	-19.9 <sup>ns</sup>	4.7 <sup>ns</sup>	0.83	Ross & Brookson(1966)
Trunk Girth	15	cm	72.4 <sup>ns</sup>	-95.9 <sup>+</sup>	0.3 <sup>ns</sup>	0.82	Ross & Brookson(1966)
Sieve Tube Size	2	$\mu$	15.3**	-1.5**	0.9**	0.46	Tan & Subramanian (1976a)
Plugging Index	2	-	11.7**	2.0 <sup>ns</sup>	0.9**	0.63	Tan & Subramanian (1976a)
Latex Vessel Number	2	-	6.8**	-0.2 <sup>ns</sup>	0.9**	0.39	Tan & Subramanian (1976a)
Latex Vessel Size	2	$\mu$	11.0**	-1.0**	0.8**	0.44	Tan & Subramanian (1976a)

+ Significant by t test ( $\alpha = 0.10$ )  
ns Non significant

\*Significant by t test ( $\alpha = 0.05$ )

\*\*Significant by t test ( $\alpha = 0.01$ )



(less than 22). This means that the diversity of those varieties is the same as the diversity among less than 22 genotypes in an ideal panmictic, unrelated and non-inbred population. Besides that, the diversity among the varieties can be even smaller than that estimated here, if it is considered that some clones recommended were planted in large areas. For example, in Malaysia, in 2002, only around 20 clones were used to plant most of the area with rubber tree in that country (1.81 million hectares) (Kong, 2002) and in 1967 around 11% of the area planted used PB 86 (Shepherd, 1969). In Bahia and São Paulo, Brazil, most of the area is planted with around 10 clones, with some of them (e.g., RRIM 600) representing a high percentage of that area.

Based on molecular markers, Seguin et al. (1995) has reported a low genetic diversity in cultivated rubber tree plantations, confirming the results found here and cited by other authors (Tan, 1987). Therefore, despite the wide variability existing within and across species in the genus *Hevea*, and the risks associated to diseases like SALB, rubber tree breeders are working with a narrow genetic base in their programs.

As shown in this study, the increased coancestry in rubber tree can result in inbreeding depression in all traits of economical importance, including yield, vigor, disease resistance and traits related to latex vessel sizes. Despite depression was not statistically significant in many of the cases studied, almost all of them had a negative impact on the trait considered.

Inbreeding depression in rubber tree has been reported also by other authors (Gilbert et al., 1973; Ross & Brookson, 1966; Sharp, 1940; Tan & Subramaniam, 1976b). Sharp (1951) observed that the vigor of crosses involving unrelated clones was larger than that involving half-sibs, and this, larger than that of full-sibs. The same trend was observed by Ross and Brookson (1966). Gilbert et al. (1973), re-analyzing the data presented by Ross and Brookson (1966), confirmed the presence of inbreeding depression. In 1976, Tan and Subramaniam (1976b) showed that selfed progenies, in the nursery, were inferior in vigor and yield than outcrossed families. However, this is the first time inbreeding depression is estimated as a function of the level of inbreeding in rubber tree.

The genetic value of the crosses considered in the study can bias the estimates of inbreeding depression either downwards or upwards. This was one of the criticisms of Ross and Brookson (1966) to Sharp (1951) conclusions, saying that the apparent depression in Sharp's study resulted from the fact that the highly inbred crosses were coincidentally those with lower genetic value. In order to clarify this, for some few groups of crosses that genetic values were available, estimates of inbreeding depression free of this effect were obtained and confirmed the occurrence of depression for some traits. Also, from these analyses, the regression coefficients associated to genetic values were always positive for the data analyzed, indicating that, as the genetic values of the parents increased, the phenotype measurements also increased. It also indicates that in a simple regression (without taking in account the genetic value of the crosses) the estimates of inbreeding depression are downwardly biased.

In this study parents of unknown origin were assumed non-inbred and unrelated, considering that the flowers of *Hevea* spp. are unisexual and the trees are monoecious and largely cross-pollinated. However, it is likely that the original plants from the Tapajós region, and used as founding clones in all three programs considered in this study, were already inbred as a result of tree isolation in the wild forest in that region, as pointed by Imle (1978), so the levels of inbreeding obtained here can be underestimated.

Increasing the diversity in the programs studied, and likely in most rubber tree breeding programs in the world, is mandatory or, to the contrary, exist the risk of limitations in future genetic gains and devastation of entire rubber plantations in some of these countries by diseases able to infect the cultivated clones. However, increasing diversity might not be an easy task. Attempts made in some of those programs have resulted in loss of gain due to the inferiority of the clones used, compared with those in use in the program (Yee, 1980).

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