INDUCTION AND INVESTIGATION OF POLYPLOIDY IN IAN 717 RUBBER TREE CLONE.

A PRELIMINARY STUDY[1]

PAULO DE SOUZA GONÇALVES[2], AFONSO CELSO C. VALOIS[3]
and JOÃO RODRIGUES DE PAIVA[2]

ABSTRACT - Buds of IAN 717 rubber tree clone were treated with a solution of colchicine plus dimethyl sulfoxide (DMSO) to induce polyploidy in plants with 2n = 36 chromosomes. Sprouts from five treated buds showed morphological variation and were vegetatively multiplied as clones. Mitotic studies of the leaf tip confirmed a high degree of mixoploidy. Morphologically, the polyploid clones differed from the control diploid in having shorter height, greater stem diameter, smaller L/B ratio of the stomata, larger petiole and larger stomata. Quantitative characters of the polyploid clones with respect to cytology, bark characters, foliage characters, stomata parameters and yield by the Mendes early test are presented. In addition, a study of the occurrence of correlation between yield and various yield determining factors of the bark was carried out. More studies on the new polyploid clones are recommended.

Index terms: *Hevea* spp., cytology, chromosomes, morphology.

INDUÇÃO E OBSERVAÇÕES DE POLIPLOIDIA NO CLONE DE SERINGUEIRA IAN 717. ESTUDO PRELIMINAR

RESUMO - Gemas do clone IAN 717 foram tratadas em solução de colchicina e Dimetil Sulfoxido (DMSO) com o objetivo de induzir poliploidia em plantas de 2n = 36 cromossomos. Brotações de cinco gemas tratadas apresentaram variações morfológicas e foram multiplicadas assexuadamente como novos clones. Estudos mitóticos do ápice foliar confirmaram um alto grau de mixoploidia. Morfologicamente, os clones poliplóides diferiram dos clones diplóides-testemunha em menor altura, maior diâmetro da haste, menor relação do comprimento e largura do estômato, maior pecíolo e maior estômato. Caracteres quantitativos dos clones poliplóides, no que diz respeito à citologia, casca, folha, estômato e produção pelo teste de Mendes, são apresentados no trabalho. Foi verificada a possível ocorrência de correlações entre produção e os parâmetros determinantes da produção da casca. Recomenda-se fazer estudos adicionais referentes aos novos poliplóides obtidos.

Termos para indexação: *Hevea* spp., citologia, cromossomos, morfologia.

INTRODUCTION

Interest in polyploidy in plants was first stimulated in 1937 when colchicine, a drug extracted from *Colchicum autumnale*, was shown to inhibit the formation of the spindle and delay the division of centromeres in plant cells. Since then, plant breeders have made numerous attempts to breed for new and superior types in crops, using this technique. They based their programs on the fact that polyploids are known to have larger cells than their diploid counterparts and it therefore seemed reasonable to expect that with larger cell and plant size, higher yields could be obtained. With increasing experience, the polyploidy technique came to be regarded as a tool capable of furnishing raw material for plant breeding programs.

The rubber tree (*Hevea brasiliensis* Muell. Arg.) is a tropical perennial plant of immense economic importance in Brazil and in Far Eastern countries. It can be propagated through either sexual or assexual means for natural rubber production.

The induction of polyploid clones in rubber tree, first reported by Ford in Sri Lanka (Rubber Research Board of Ceylon 1942), was unsuccessful. Research on polyploidy in *Hevea* has been done by Mendes & Mendes et al. (1963) in Brazil, Shepherd (1969) in Malaysia and Markose (1975) in India. Their initial results created great interest because of the increased vigor of resultant plants. Subsequent developments of this earlier work have not however been reported.

[1] Accepted for publication on July 15, 1983.
This research has been supported by a SUDHEVEA/EMBRAPA agreement.

[2] Plant breeder, M.Sc., EMBRAPA - Centro Nacional de Pesquisa de Seringueira e Dendê (CNPSD), Caixa Postal 319, CEP 69000 - Manaus, AM - Brazil.

[3] Plant breeder, Ph.D., EMBRAPA-CNPSD.

Mendes (1971) and Pinheiro et al. (1980) reported an increase in yield in several tetraploid clones in the immature stage, utilizing the micro-tapping test. Chimeral problems have been observed and quality improvement is receiving attention to improve the technique and reduce this problem (Moraes 1982).

In the present study, attempts were made to induce polyploidy in *Hevea* by treating vegetative buds with colchicine. Detailed preliminary studies were also conducted on the characteristics utilized for polyploidy identification.

MATERIAL AND METHODS

Several ramets of IAN 717 clone were selected for treatment with colchicine. This clone, a primary hybrid clone of a *Hevea benthamiana* and *Hevea brasiliensis* cross was chosen because of its high yield and SALB tolerance and is widely used in rubber plantations in northern Brazil.

The technique for polyploid induction in *Hevea* essentially follows that of Mendes (1977a), modified by Empresa Brasileira de Pesquisa Agropecuária (1978). Mature budded one-year-old remets with $2n = 36$ chromosomes were stumped at a suitable height in order to force the sprouting of dormant buds. Very young sprouts (1-2 mm size) were rubbed with a very fine sandpaper in order to obtain a more direct contact of colchicine solution with the various meristematic cell layers. Two hours after the operation, the fine layer of coagulated latex found on the buds was removed. The sprouts were treated with a solution of 0.375% colchicine plus 0.125% dimethyl sulfoxide, added to the colchicine solution to induce a rapid contact of the colchicine with the sprout tissue. The aqueous solution was placed in contact with the sprout through an apparatus made from a small piece of plastic tube fixed to the plant at the point of the sprouting bud. In order to prevent leakage of the solution, the plastic tube was plugged with beeswax. Five mililiters of the solution were poured into the apparatus. After two hours the device was removed but the wax was left on the treated bud as an indication of treatment.

For each ramet, young sprouts were used, while one bud was left untreated as a check.

Seven months later, branches that showed sings of ploidy were removed, leaving 15 cm of stump. The new shoots were again observed for ploidy characters. The brances that continued to show these characters were grafted and raised as five new clones.

One year later, identification of ploidy was made by chromosome count of primordial leaf cells obtained from apical shoots for ramets from each clone. The Feulgen reaction technique, modified (Rebello 1981), was employed for this study.

Yield, leaf thickness, weight of leaf sample, stem index, petiolar index and foliar index measurements were taken as follows:

a. yield was estimated by use of the early test utilized in Brazil (Mendes 1971) for testing young plants;
b. thickness of mature leaf was based on an average of ten leaves per plant and measurements were taken with a micrometer;
c. weight of the leaf sample was expressed in miligrams per square milimeter;
d. stem index (I_s) was expressed by the ratio $I = 100\ D/L$, where D = stem diameter of the petiole (measured in the middle of its total length) and L = length of the petiole;
e. foliar index (I_f) was expressed by the ratio $I_f = 100\ D_f/L_f$, where D_f = diameter of the principal vein (measured in the middle of its total length) and L_p = length of the principal vein.

Stomata distribution and size of guard cells were recorded, utilizing the method followed by Balbach et al. (1977). Only mature leaves collected at 8-9:00 A.M. and examined immediately were utilized in this study.

Characters of the bark, including thickness, total number of latex vessel rings, density of latex vessels per mm^2 per ring, and diameter of latex vessels were included. The bark parameters were measured as follows:

a. bark thickness was measured in the laboratory from samples taken at 25 cm from the ground;
b. latex vessel numbers were determined by examining the radial longitudinal sections of the same bark samples;
c. latex vessel diameter was observed in the transverse section of the bark samples;
d. density of latex vessels per mm^2 was determined by the average density bases on all rings.

RESULTS AND DISCUSSION

Five branches from buds treated with colchicine solution were found to have a degree of ploidy. The mosaic appearance of the leaves was due to the presence of both diploid and polyploid tissues. The polyploid clones differed morphologically from diploid ones by a darker green leaf color and more compact growth in the juvenile stage.

Cytology

Leaf chromosomes from all ramets from different clones, including the normal and polyploid ones, were counted in order to determine the degree of ploidy. Leaf cell metaphase was studied one year after field transplant. In all ramets, leaf cells revealed a considerable variation in chromosome number (Table 1), with most cells having chromosome counts between 36 and 72. Clones PS 1 had many leaf cells with chromosome numbers between 53 and 72, and relatively few cells with 36 chromosomes. This indicated a high degree of ploidy in chromosome numbers with few diploid number cells.

In Table 1 the results indicate an irregularity in cell chromosome number compared to the true polyploid, an intriguing problem which will require further study.

To confirm this fact, Chen et al. (1982) observed a considerable variation in chromosome number at metaphase of leaf cells of haploid plantlets. A possible explanation is that in polyploidy induction in trees, the meristematic tissue of growing buds consists of two self--perpetuating tissues, the outer tunica and the inner corpus, which consist of several dividing cells. According to the authors, it is difficult to obtain a concentration which will cause simultaneous chromosome doubling and diffusion of colchicine solution to the interior meristematic cells in all cells or both layers-(Wright 1976). In *Hevea*, according to Markose (1975), it is possible that the absorption and diffusion of colchicine solution to the inner corpus may not occur and only the outer cells are affected.

Bark characters

Quantitative studies on polyploid clones determining bark thickness, total number of latex vessel rings, diameter of latex vessels, and density of latex vessels per 5 mm of ring are given in Table 2. Except for total number or latex vessel rings, all other characters measured confirm the thesis that polyploid materials are generally superior to diploid materials. The latex vessels in the bark were larger and numerically less compared to diploid plants. Similar findings were recorded by Markose et al. (1977) in clones of polyploids at the Rubber Research Institute of India.

Average standard deviation and coefficient of variation relating to leaf thickness, weight per leaf sample, stem petiolar and foliar indexes are shown in Table 3.

According to Lleras & Medri (1978), the epidermal and palisade cells in the leaves of the polyploid plants were larger in size compared to the diploid plants. The increase in cell size contributed to the increase in leaf thickness and weight per unit area. Mendes (1977b) reported similar findings on clones of polyploids obtained from IAN 873 clone, an intraspecific cross of *Hevea brasiliensis*. The stem, petiolar and foliar indices in general were high in the polyploid clones. Except for the petiolar index, higher values were obtained for clone PS 05, with values of 11.54, 2.020 and 0.740, respectively.

Stomata distributions

According to Markose (1975) and Mendes (1977b), the reduced number of stomata per unit area and larger size are characters employed for polyploid identification. The results are confirmed in Table 4 for the five polyploid clones. In other species, it has been found that polyploid epidermal

TABLE 1. Chromosome counts in metaphases of young leaf cells from colchicine treated bud from five polyploid *Hevea* clones. Manaus, AM, 1982.

Clone	Average height of plant (m)	Number of leaves	Number of metaphases	Chromosome counts	
				35-54	55-72
IAN 717[1]	1.20	15	42	40	2
PS 01	0.81	8	45	6	39
PS 02	0.92	10	23	5	18
PS 03	0.75	12	33	5	28
PS 04	0.86	6	22	4	18
PS 05	0.95	2	20	7	13

[1] Diploid control clone.

TABLE 2. Number of investigated ramets (n), mean ($\bar{x}$), standard deviation (s) and coefficient of variation (C.V.) of four bark characters obtained from five polyploid clones. Manaus, AM, 1982.

Clone	Bark thickness (mm)	Total number of latex vessel rings	Diameter of latex vessel (μ m)	Density of latex vessels per 5 mm per ring
Diploid control clone				
IAN 717				
n	15	15	15	15
x	1.080	1.000	14.800	63.930
s	0.173	0.000	1.300	6.130
C.V.	16.632	0.000	8.770	9.598
PS 01				
n	8	8	8	8
$\bar{x}$	1.237	1.000	16.866	64.536
s	0.172	0.000	0.802	6.410
C.V.	13.965	0.000	4.759	9.933
PS 02				
n	10	10	10	10
$\bar{x}$	1.163	1.000	14.185	62.290
s	0.228	0.000	1.670	5.560
C.V.	19.682	0.000	11.776	10.531
PS 03				
n	12	12	12	12
$\bar{x}$	1.100	1.000	15.628	61.470
s	0.152	0.000	1.099	4.100
C.V.	13.886	0.000	7.037	6.669
PS 04				
n	6	6	6	6
$\bar{x}$	1.166	1.000	16.898	69.668
s	0.047	0.000	0.924	6.259
C.V.	4.042	0.000	5.472	8.984
PS 05				
n	1	1	1	1
$\bar{x}$	1.60	1.000	14.82	140.000
s	-	-	-	-
C.V.	-	-	-	-

tissues invariably have fewer stomata and larger cells than diploids (Shepherd 1969). The L/B ratio of length and breadth of the stomata was found to be smaller in the polyploids than in the diploid control (Table 4). Similar results have been reported in autotetraploids from the GT 1 rubber tree clone (Markose 1975).

Yield

The average latex yield in miligrams per tapping per clone in three early tests rounds, except for clones PS 05, was significantly higher for the four clones (Table 5) than for the diploid control clone IAN 717. Other studies carried out by Pinheiro et al. (1980) and Mendes (1971) showed that for one year, IAN polyploid clones produced high yields when compared with the diploid control clone IAN 873.

The high coefficient of variation may be attributable to the interaction of the diploid

TABLE 3. Number of investigated ramets (n), mean ($\bar{x}$), standard deviation (s) and coefficient of variation (C.V.) of five foliage characters obtained from five polyploids clones. Manaus, AM, 1982.

Clone	Thickness of leaf blade (mm)	Weight of leaf sample (mg)	Stem index	Petiolar index	Foliar index
Diploid control clone					
IAN 717					
n	10	10	10	10	10
$\bar{x}$	0.198	16.130	6.130	1.383	0.570
s	0.012	0.235	0.120	0.120	0.040
C.V.	6.060	1.456	1.957	8.676	7.010
PS 01					
n	8	8	8	8	8
$\bar{x}$	0.246	22.053	9.405	1.890	0.712
s	0.019	2.281	1.794	0.242	0.074
C.V.	7.723	10.343	19.083	12.804	10.504
PS 02					
n	10	10	10	10	10
$\bar{x}$	0.237	22.484	7.275	1.851	0.650
s	0.009	2.583	1.802	0.085	0.032
C.V.	3.797	11.489	24.777	4.357	4.961
PS 03					
n	17	17	17	17	17
$\bar{x}$	0.262	21.007	8.811	1.892	0.719
s	0.051	2.564	3.014	0.215	0.067
C.V.	19.465	12.208	34.210	11.409	9.450
PS 04					
n	6	6	6	6	6
$\bar{x}$	0.263	21.278	6.445	2.053	0.730
s	0.008	2.333	0.369	0.039	0.062
C.V.	3.041	10.967	5.736	1.941	8.627
PS 05					
n	1	1	1	1	1
$\bar{x}$	2.910	23.300	11.540	2.020	0.740
s	-	-	-	-	-
C.V.	-	-	-	-	-

rootstock on which the polyploid material was grafted, contributing to the different leaf flush stages during the time that the early test was done in the clone ramets. Similar values were obtained by Gonçalves et al. (1982). According to Paranjothy (1980), low yields are most accentuated during new foliage production, undoubtedly caused by demand for mineral nutrients and carbon by the developing leaves.

However, this high variation would suggest the need for propagation of polyploid clones by root cuttings. The importance of this approach is that it could avoid the possible adverse interaction between the polyploid scion and the diploid rootstock.

Yield-bark characteristics relationship

Using the bark characters of the polyploid clone ramets as defined, coefficients of correlation were determined (Table 5). The study was carried

TABLE 4. Number of investigated ramets (n), mean (x) standard deviation (s) and coefficient of variation (C.V.) of four stomata parameters obtained from five polyploid clones. Manaus, AM, 1982.

Clone	Density of stomata per mm^2	Breadth (μ)	Length (μ)	Length/Breadth ratio (μ)
Diploid control clone				
IAN 717				
n	10	10	10	10
$\bar{x}$	329.156	21.323	34.983	1.643
s	10.163	2.325	2.233	0.053
C.V.	3.087	10.903	6.383	3.225
PS 01				
n	8	8	8	8
$\bar{x}$	232.250	25.632	40.208	1.588
s	31.530	4.165	2.850	0.134
C.V.	13.570	16.250	7.089	8.439
PS 02				
n	10	10	10	10
$\bar{x}$	240.000	24.526	38.226	1.561
s	8.763	1.467	1.077	0.080
C.V.	3.651	5.981	2.817	5.184
PS 03				
n	17	17	17	17
$\bar{x}$	215.352	29.103	43.146	1.494
s	34.850	2.491	3.782	0.170
C.V.	16.183	8.560	8.766	11.417
PS 04				
n	6	6	6	6
$\bar{x}$	271.666	26.738	39.243	1.473
s	31.008	1.113	2.333	0.135
C.V.	11.414	5.652	5.947	9.214
PS 05				
n	1	1	1	1
$\bar{x}$	237.000	24.400	46.770	1.920
s	-	-	-	-
C.V.	-	-	-	-

out to examine the existence of correlation between yield and various yield determining factors of the bark. Significant results were not found for the correlation of yield with bark thickness (r = 0.2214), diameter of latex vessels (r = 0.1389) and density of latex vessels per 5 mm (r = 0.1192), although several investigators (Wycherley 1969, Narayanan et al. 1973, Ashplant 1928, and Gonçalves et al. 1980) have shown that there is a positive correlation between yield and bark characters in diploid materials. As pointed out, this is not evident for the polyploids in the present study, which may be due to the level of mixoploidy obtained. More lengthy studies should therefore be made on ramets. The mixoploidy might be reduced gradually and totally disappear as a branch or a tree becomes wholly polyploid or diploid (Wright 1976).

TABLE 5. Number of investigated ramets (n), mean ($\bar{x}$) standard deviation (s) and coefficient of variation (C.V.) of three MTP[1] rounds test on polyploid clones. Manaus, AM, 1982.

Clone	1st MTP	2nd MTP	3th MTP	Mean (mg)
Diploid control clone				
IAN 717				
n	15	15	15	15
$\bar{x}$	10.044	17.903	23.197	17.048
s	8.053	8.852	7.884	7.169
C.V.	80.177	49.444	33.987	42.071
PS 01				
n	9	9	9	9
$\bar{x}$	10.976	20.50	23.830	18.430
s	5.120	6.36	6.123	4.621
C.V.	46.647	31.02	25.690	25.663
PS 02				
n	10	10	10	10
$\bar{x}$	24.501	23.122	38.652	26.244
s	4.958	7.521	19.445	7.313
C.V.	20.204	33.997	50.307	27.865
PS 03				
n	18	18	18	18
$\bar{x}$	17.180	18.825	22.920	19.305
s	1.519	2.892	2.250	2.313
C.V.	8.841	15.360	9.816	11.981
PS 04				
n	8	8	8	8
$\bar{x}$	14.985	23.407	37.858	24.640
s	3.900	11.256	37.883	16.780
C.V.	26.026	48.088	50.089	6.810
PS 05				
n	1	1	1	1
$\bar{x}$	4.260	9.940	12.060	8.753
s	-	-	-	-
C.V.	-	-	-	-

[1] MTP - Microtapping.

TABLE 6. Linear correlation coefficients among various yielding determining factors of the bark. Manaus, AM, 1982.

Parameters	Symbol	Y	B	DVL	DVR
Yield/t/t	Y	1	0.2214 n.s.	0.1389 n.s.	0.1192 n.s.
Bark tickness	B		1	0.5712 *	0.6731 ***
Diameter of latex vessels	D.V.L.			1	0.77066 ***
Density of latex vessels per 5 mm	D.V.R.				1

* $P < 0.05$ - significant at 5% level of probability.
*** $P < 0.005$ - significant at 0.5% level of probability.

CONCLUSIONS

1. The principal observations made on the polyploid clones were: reduction in initial growth, mosaic leaflet thickness and dark green leaves.

2. Morphological characteristics of bark, leaf stomata and cytology revealed a high level of ploidy in the induced material.

3. Anatomical investigation of the clones revealed that most of the colchicine treated ramets have thick bark, larger stomatal size and fewer number of stomata in a unit area.

4. Cytological investigations revealed a high incidence of mixoploidy in the leaf tissue of the induced clones.

5. Yield by macro-tapping showed that the polyploid clones had superior yield in comparison with the check clone IAN 717.

6. No significant results were found for correlation of yield with various yield determining factors of the bark.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Antonio Pessoa Rebello and Mr. José Arinos Nascimento Araújo for their laboratory and field work, respectively.

REFERENCES

ASHPLANT, H. Investigations into *Hevea* anatomy. Bull. Rubber Grow Assoc., 10(7):484, 1928.

BALBACH, M.L.; BLISS, L. & FULLER, M.J. A laboratory manual for general botany. 5.ed. New York, Holt, Rinehart and Winston, 1977. 393p.

CHEN, Z.; QUIAN, C.; QIN, M.; XU, X. & XIAO, Y. Recent advances in anther culture of *Hevea brasiliensis* (Muell Arg.). Theor. Appl. Genet., 62: 103-8, 1982.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. Centro Nacional de Pesquisa de Seringueira e Dendê, Manaus, AM. Poliploidização da seringueira. Relatório anual, 1978, Manaus, 1978. p.43.

GONÇALVES, P. de S.; PAIVA, J.R. de & ROSSETTI, A.G. Eficiência do miniteste de produção (MTP) e sua relação com o teste Hamaker Morris-Mann (HMM) na seleção precoce de plantas de seringueira. Pesq. agropec. bras., Brasília, 17(8):1145-53, 1982.

GONÇALVES, P. de S.; VASCONCELLOS, M.E.C.; VALOIS, A.C.C. & BARCELOS, E.S. Herdabilidade, correlações genéticas e fenotípicas de algumas características de clones de seringueira (*Hevea* spp.). Pesq. agropec. bras., Brasília, 15(2):129-36, 1980.

LLERAS, E. & MEDRI, M.E. Comparação anatômica entre folhas diplóides e poliplóides do híbrido *Hevea brasiliensis* x *benthamiana* (IAN 717). Acta amaz., 8(4):565-75, 1978.

MARKOSE, V.C. Colchyploidy in *Hevea brasiliensis* (Muell. Arg.). Rubb. Board Bull., 12(1):3-5, 1975.

MARKOSE, V.C.; PANIKKAR, A.O.N.; ANNAMMA, Y. & NAIR, B.V.K. Effect of gamma rays on rubber seed germination seedling growth and morphology. J. Rubb. Res. Inst. Sri Lanka, Ceylon, 54:50-64, 1977.

MENDES, L.O.T. Miniteste de produção para seringueiras jovens. Polímeros, Rio de Janeiro, 1(1), jan./mar. 1971.

MENDES, L.O.T. Técnica para poliploidização da seringueira I. Elastômeros, 3:3-10, 1977a.

MENDES, L.O.T. Técnica para poliploidização da seringueira II. Elastômeros, 4:2-11, 1977b.

MENDES, L.O.T. & MENDES, A.J.T. Poliploidia artificial em seringueira. Bragantia, 22(30):383-92, 1963.

MORAES, V.H.F. Organogênese em meristema apical do caule de seringueira (*Hevea* spp.). Pesq. agropec. bras., Brasília, 17(6):893-4, 1982.

NARAYANAN, R.; GOMEZ, J.B. & CHEN, L.T. Some structural factors affecting the production of *Hevea brasiliensis*. II Correlation studies between structural factors and yield. J. Rubb. Res. Inst., Malaya, 23(4): 285, 1973.

PARANJOTHY, K. Physiological aspects of wintering, flower induction and fruit-set in *Hevea*. In: HEVEA BREEDING COURSE. Kuala Lumpur, RRIM, 1980. 12p.

PINHEIRO, F.S.V.; PINHEIRO, E. & CONCEIÇÃO, H.E.O. da. Avaliação de clones poliplóides de seringueira (*Hevea* sp.) da série IAC. In: SEMINÁRIO NACIONAL DA SERINGUEIRA, 3, Manaus, 1980. Anais... s.l., SUDHEVEA, 1980.

REBELLO, A.P. Pesquisas técnicas de contagem de cromossomos. Manaus, EMBRAPA-CNPSD, 1981. 10p.

RUBBER RESEARCH BOARD OF CEYLON, Ceylon, Sri Lanka. Report of the Botanical and Micological Department. In: ________. Report of the work of the Rubber Research Board in 1941. s.l., Rubber Research of Ceylon, 1942. 10p.

SHEPHERD, R. Induction of polyploid in *Hevea brasiliensis*. Preliminary observations on trials conducted at Prang Besar Rubber Research Station. Plant. Bull. Rubb. Res. Inst. Malaysia, 104:248-56, 1969.

WRIGHT, J.W. Introduction to forest genetics. London, Academic Press Inc., 1976. 463p.

WYCHERLEY, P.R. Breeding in *Hevea*. J. Rubb. Res. Malaya, 21(1):38-55, 1969.