PROGRESS REPORT NO.6

" BIOTECHNOLOGY APPLICATION FOR CHARACTERIZATION AND SELECTION OF BETTER-YIELDING RUBBER CLONES"

A RESEARCH PROJECT

USAID/PSTC PROGRAM

Grant No. 936-5542-G-00-6054-00

Submitted by

Dr. Rapepun Wititsuwannakul

Project Leader

Department of Biochemistry

Faculty of Science

Prince of Songkla University

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Project Profile

Country	:	Thailand
Grant No.	:	936-5542-G-00-6054-00
Program	:	Program on Science and Technology Cooperation
Project Title	:	Biotechnology Application for Characterization and Selection of Better-Yielding Rubber Clones.
Project Leader	:	Dr. Rapepun Wititsuwannakul
Organization	:	Dept. of Biochemistry, Faculty of Science, Prince of Songkla University, Hat-Yai 90112, Thailand.
CO- investigators	:	Dr. Wallie Suvachitanont, Dr. Rapiporn Sotthibandhu, Dr. Dhirayos Wititsuwannakul, Mr. Apichai Shuprisha, Mr. Wisut Sukonrat. Dr. Nunta Churngchow
Authorized Officer	:	Dr. Phasook Kullavanij (President)
Total Project Budget	:	US $ 150,000
Project Duration	:	3 years (27 June-1986-31 Deccember 1989)
Reporting Period	:	1 January-30 June 1989

Budget Allocation for This Period : US $ 291,882

1. Background/Introductions :

Part A. Biochemical parameters for rubber yield evaluation in *Hevea brasiliensis*.

Traditional practice on yield evaluation of rubber is to grow the plant for at least six years until trees can be tapped and yield measured. Early attempt on yield evaluation studying relation between latex yield of *Hevea* and rubber biosynthesis in *vitro* was reported in 1965 by D'Auzac (1). The study involved latex specimens chosen from pairs of high-and low-yielding trees growing in a monoclonal plot. The efficiency of rubber biosynthesis from 2-^{14}C acetete was shown distinctly higher in the high yielders. In 1970, C.H. Woo and E.E. Edwin performed similar incorporation experiment using trees from different clones and chosen at random (2). The results led to similar conclusion but large variations made it difficult to make reliable distinction of high and low-yielding potentials. Our studies on diurnal variation of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) activity showed the enzyme was closely related to rubber content in the latex (3). The correlation suggested possible role of this enzyme in regulation of rubber biosynthesis. In this study we proposed to test and verify HMGR as a rate limiting enzyme in rubber biosynthesis pathway. Its possible use as an enzyme parameter in yield evaluation will be further pursued and utilized in clonal selection processes.

Part B. Disease resistance screening of *Hevea* at tissue cluture stage

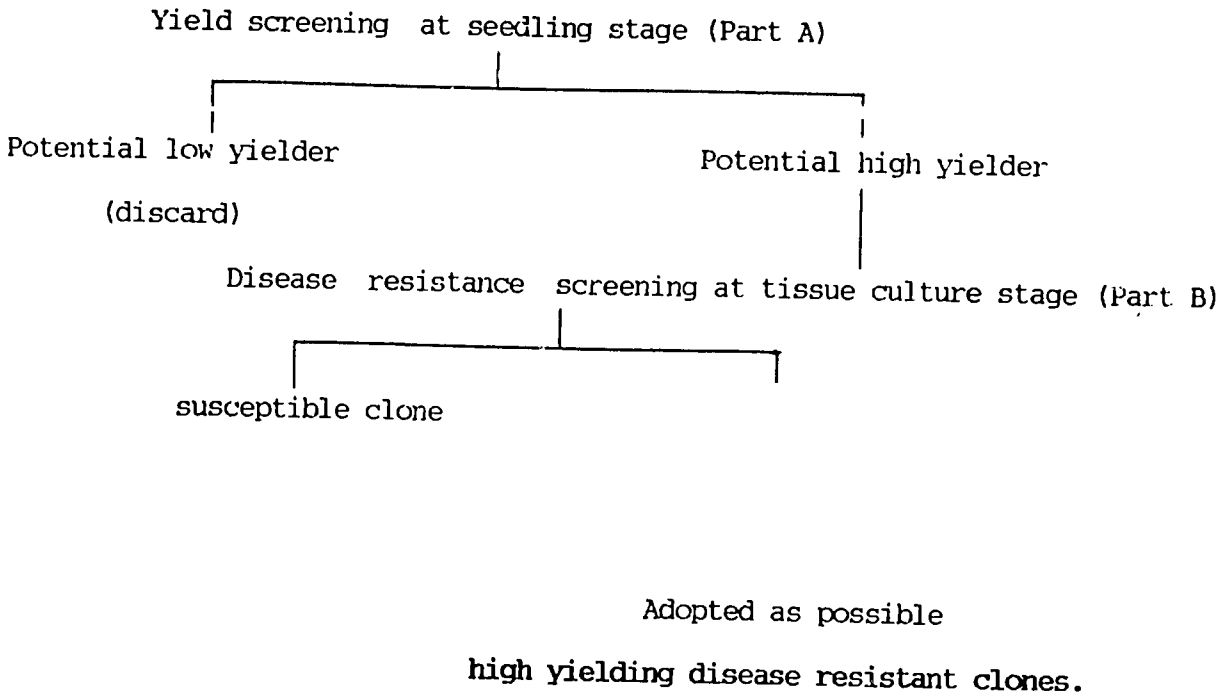
Since *Hevea brasiliensis* is a perennial tree crops and thus the duration of breeding cycle is rather long. It takes 5-6 years for *Hevea*

to grow before the first tapping can be commenced, after which it can be tapped for rubber latex for a period of 30 or more years. It is, therefore, of great importance to select for a disease resistant clones in addition to the high-yielding potential concurrently. This is an added advantage to prevent growth and rubber production failure due to disease or disturbance by the pathogens. We proposed a system to study the disease resistance at tissue culture level in this study as an early selection in conjunction with high-yielding potentials.

2. Objectives :

A. To develop a reliable method for identification and selection of higher yielding rubber clones at an early stage, while the plants are still at seedling stage. This would help circumventing the time and energy needed in conventional approaches.

B. To initiate the study of disease resistant rubber clones at the level of tissue culture stage in conjunction to the high-yielding potential screening as outlined below.



Materials and methods :

Part A. Biochemical parameter for rubber yield evaluation in *Hevea brasiliensis.*

Chemicals :

All chemicals and reagents used were of analytical grade. DTT NADPH, HMG-CoA, mevalonolactone, acetyl-CoA, acetoacetyl CoA, ATP, were from Sigma. DL-2-^{3}H mevalonate,1-^{14}C acetyl-CoA, DL-3^{14}C HMG-CoA were from Amersham England.

Collection of latex :

Latex was obtained from young buddings (8 mo. old after grafted on 4 yr. old stock) of various clones of new *Hevea* germplasm by tapping at

0600 h. After tapping, 1 ml of fresh latex was collected and dutuled 1:1 (v/v) with buffer X before used in HMGR assay.

Collection of leaf samples :

Young rubber seedlings approximately 6 month-old of clones RRIM 600, PB 235, and BPM 24 were illiuminated (6,000 lux for 12 hours, 0600-1800 h) daily in experimental control room. The plants were allowed to adapt to the new environment for one month before used for experiments. After the adaptation period, newly mature leaves were detached from the young rubber plant and used for determination of HMGR, MVA kinase, HMG-CoA synthetase, and petiole rubber value.

Determination of petiole rubber value :

The petiole latex method of Zhou Zhongyu et al. (4) was used for petiole rubber value determination. Rubber latex exuded from newly detached leaf was smeared on a preweighed weighing paper. The sample was dried at 60-70°C until constant weight obtained. The petiole rubber value was determined as followed.

$$A = \frac{R_1 - R_2}{W_1}$$

A = petiole rubber value

R_1 = dry latex smear + paper weight

R_2 = paper weight

W = dry leaf weight

Preparation of leaf homogenates :

The middle leaflet of *Hevea* compound leaf was picked from young

rubber seedlings kept under 6,000 lux of fluorescent light (a mixture of Philips Daylight TL 33 and TL 54 and Mazdafluor AVIVA). After removal of the midribs, the leaf tissues were cut into small pieces and finely ground in a mortar in the presence of liquid nitrogen, then homogenized in three volumes of extraction medium (0.1 M potassium phosphate buffer pH 7.0 containing 1 mM EDTA and 14 mM 2-mercaptoethanol) for one minute (2 pulses per 30 seconds) with a Polytron homogenizer. The crude homogenate was used for enzyme assays after filtration through 6 layers of gauze.

Assay of HMG-CoA reductase (HMGR) activity :

The HMGR acctivity was assayed in buffer X (0.1 M KCl, 10 mM DTT 30 mM EDTA, 0.1 m K_xPO_4, pH 7.0) containing fresh latex with protein ranged from 0.1-1.8 mg, 0.4 umoles NADPH, 120 nmoles DL-$3^{14}C$ HMG-CoA (1000 dpm/ nmole) in a final volume of 50-100 ul. The incubation was run at 37°C for 20 or 30 min as indicated. The reaction was stopped by addition of 25 ul of 10 N HCl. Unlabeled mevalonolactone was then added, and the mixture was allowed to incubate for another 30 min for complete lactonizaion of the incubation product (mevalonic acid). The precipitated protein was removed by centrifugation and the product of the enzyme was purified by TLC using benzene acetone (1:1 v/v) as solvent system and developed under iodine vapor according to the method of Iijima et al. (5). The zone of mevalonolactone was scraped into a scintillation vial containing 10 ml of dioxane fluor. Samples were allowed to stabilize overnight before counting in Packard liquid scintillation counter.

Assay of MVA kinase :

MVA kinase activity of leaf homogenates were assayed by a radiochemical procedure which was modified from that of Gray and Kekwick (6). Incubation mixtures contained DL-[2-^{3}H]-mevalonate (1.025 umol, sp. radioactivity 0.15 uci/mol), ATP (2 umol), $MgCl_2$ (1 umol) and 0.05 ml of leaf homogenate in 50 mM potassium phosphate buffer pH 7.0, in a total volume of 0.2 ml. Reactions were started by addition of mevalonate and ATP after the preincubation of the assay mixture at 30°C. The reaction was allowed to proceed for 10 minutes and stopped by boiling for 2 minutes. The proteins were removed by centrifugation. Portions of the supernatant (100-150 ul) were spotted on Whatman 3MM filter paper strips (2.5 x 22 cm). Descending chromatographic separation was carried out with butan-1-ol : formic acid : water (77 : 10 : 13 by volume). The paper sections containing the radioactive product (mevalonate 5-phosphate) were cut out between R_f 0.12-0.25 (7) and counted in the liquid scintillation counter using toluene-dioxane cocktail.

Assay of HMG-CoA synthetase activity :

The assay of HMG-CoA synthetase in leaf homogenate was carried out by following the incorporation of 1-^{14}C-acetyl-CoA in HMG-CoA or its dehydration product, 3 methylglutaryl-CoA. The reaction mixture was in 1 M Tris-HCl, pH 8.2 and contained 0.1 mM EDTA, 0.2 mM 1-^{14}C- acetyl-CoA (4 x10^5 cpm/umole), 0.05 mM acetoacetyl-CoA, and leaf homogenate (50 ul) in a final volume of 0.2 ml. (8). The reaction was initiated by adding ^{14}C-acetyl-CoA to the preincubated reaction mixture (2 min at 30°C). At 2, 4, 6 and 8 minutes after ^{14}C-acetyl-CoA addition, 40 ul aliquots are transferred into glass vials containing 0.1 ml 6 N HCl. The

acidified aliquots were taken to dryness at 95°C using heating plate. Water (0.5 ml) is added to dissolve the dried nonvolatile material. Liquid scintillation cocktail; compatible with 10% final H_2O content, was then added. The labelled nonvolatile material, HMG-CoA or its dehydration product, was determined in liquid scintillation counter. Correction for and nonvolatite ^{14}C-acetyl-CoA was determined by using controls in which leaf homogenate was omitted.

Protein determination :

The protein concentration was determined by the method of Lowry et al. (9).

Preparation of antibody against HMGR :

Rabbit immunization

Approximately 50 ug of purified HMGR in 0.5 ml of complete Frend's adjuvant was prepared for each injection dose. The protein antigen mixture was injected subcutaneously to rabbits at 1 week interval. The titer of antibody was tested at least 4 days after the third injection.

Immunotitration of enzyme

Antibody against HMGR was test for specificity using Ouchterlony immunodiffusion against purified HMGR as well as HMGR in fresh latex. Various amount serum from immunized rabbit was incubated with purified HMGR in fresh latex to test for its ability to inhibit the HMGR activity in fresh latex using preimmunized rabbit serum as a control.

4. Result/Discussions/Tables :

Part A. Biochemical parameter for evaluation of rubber yield in *Hevea brasiliensis*.

A.1 Diurnal variation of leaf HMGR activity and its relation to petiole rubber value

Our previous report on diurnal variation of HMGR activity in rubber latex and its relation to rubber content (report 1) revealed that they were closely related. A similar study on diurnal variation of HMGR activity in leaves and its relation to petiole rubber value was performed (Fig.1 and 2) in this report. It is seen that there was a high correlation between leaf HMGR activity and petiole rubber value in both clonal types studied. Both HMGR activity and petiole rubber values reached their maximum at 1800 h. At this time, the average value on difference between the highest and lowest levels of HMGR activity and petiole rubber values were 5.5 and 3.5 fold, respectively. The amount of fold increase on enzymic specific activity level is only 1.5 fold higher than that of petiole rubber value. This suggest a close relationship between HMGR activity and petiole rubber value levels.

A.2 Diurnal varriation of leaf MVA kinase activity and its relation to petiole rubber value

Three clonal types namely RRIM 600, PB 235 and PBM 24 were used in this study. A postitive correlation between MVA kinase activity and petiole rubber value was observed in clones PB 235 and RRIM 600 (Fig.3 and 4). The maximum levels of MVA kinase for PB 235 and RRIM 600

were reached at 1800 and 2200 h, respectively. At the times mentioned, the average values on differences between the highest and lowest levels of MVA kinase activity and petiole rubber value were 11.4 and 2.1-fold, respectively. The amount of fold-increase in enzymic specific activity is much higher (5.4 -fold) than that of petiole rubber value. Therefore, the close MVA kinase activity and petiole rubber value level is unlikely to exist.

A.3 Diurnal variation of leaf HMG-CoA synthetase activity and its relation to petiole rubber value

There was clearly no positive correlation between HMG-CoA synthetase activity (Fig.5) and petiole rubber value (Fig.4) from three *Hevea* clonal types (RRIM 600, PB 235 and PBM 24) used in the study. The maximum change on HMG-CoA synthetase activity level was observed in clone BPM 24 at 1800 h with 6.3-fold increased from its basal level while there is only a slight increase in petiole rubber value. This result suggests an unrelated relationship between HMG-CoA synthetase and peticle rubber value.

A.4 Preparation of antibody against HMGR

The antibody was obtained from immunized rabbit serum after one week following the third injection. The titer of rabbit antiserum is 1:10 and higher than last report (no.5). The test for the ability of HMGR antibody on HMGR activity inhibition is still not successful.

A.5 Preliminary yield screening on new *Hevea* germplasms

An additional preliminary yield screening on new *Hevea* germplasms were carried out on leaf latex measurements. The yield was

evaluated on the amount of latex obtained upon cutting through leaflet of 3 months old seedings using methods of Zhongu, et al. (4). Leaf blade was cut parallel on both sides of midrib vein with 1 cm apart. The amount of latex being excreted from lateral veins were then measured visually. Grades A to D were given for high to low latex yield obtained. Results on yield evaluation by estimating latex being excreted from lateral veins are shown in Table I. There were 26 new germplasms which demonstrate high yield potential with grades A. They were subsequently subjected to further yield evaluation by comparing their latex HMGR levels(obtained from stem-microtapping) to that of RRIM 600 under similar treatment. Results on latex HMGR levels on RRIM 600 and 26 new garmplasms are shown in Table II. Most of the potential high yielders have lower latex HMGR activity than that of RRIM 600 which is the established known high yielder. There are, however, 8 new clonal types coded MT/C/2(10/27), RO/A/7(25/500). RO/PB/2(3/287), AC/3/8(35/518), RO/A/7(25/539), MT/C/4 (7/135), MT/I/21(24) and RO/A/7(25/519) where latex HMGR activity are higher than that of RRIM 600. The highest HMGR activity level was obtained from clone under MT/C/2 (10/27) coding and about 6 times higher than that of RRIM 600.

5. Conclusion remarks :

-The leaf diurnal studies confirmed a close relationship between HMGR activity and petiole rubber value.

-The MVA kinase also exhibited a positive diurnal corelation with the petiole rubber value but with a lesser extent when compared to those observed between the HMGR. and petiole rubber value.

-The positive corelation between HMG-CoA synthetase and the petiole rubber value was not observed in the diurnal studies.

-Several high yield potentials were identified from the new *Hevea* germplasm screening using the HMGR as a yield parameter.

6. Work plan for next period

-Continuation on the ELISA technique development for HMGR measurement.

7. Reference

1. J.D'Auzac (1965) Relations entre la composition chimique du latex, 1' intensite de quelques reaction metaboliques et la productivite de 1' *Hevea brasiliensis.* Revue gen. Caoutch, Plastiq. *42 (7-8)* 1027.

2. C.H. Woo and E.E. Edwin (1970) Relationship between yield of *Hevea* and rubber biosynthesis *in vitro.* J. Rubb. Res. Inst. Malaya *23,* 68.

3. R. Wititsuwannakul (1986) Diurnal variation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in latex of *Hevea brasiliensis* and its relation to rubber content. Experientia *42,* 44.

4. Z. Zhongyu, Y. Xiehui, W. Lizhen, G. Qiyuan, H. Xiang, Z. Sairong C. Chuangin and L. Jinxing (1984) Approaches to early prediciting mature rubber yield potential at juvenile stage and their theoretical basis. Paper from South China Academy of Tropical Crops, presented at IRRDB Conference, Beijing, China.

5. Y. Iijima and M. Maruyama (1973) Simplification of the assay

of 3-hydroxy-3-methylglutaryl CoA reductase activity. 15th. Proceedings of Japan Council of Biochemistry of Lipids *15,* 164.

6. J.C. Gray and R.G.O. Kekwich (1973) mevalonate kinase in green leaves and etiolated cotyledons of French bean *Phaseolus valgaris.* Biochem. J. *133,* 335.
7. T.T. Tchen (1962) Enzymes in sterol biogenesis. In "Methods in Enzymology" vol. 5 pp. 489-499. S.P. Colowick and N.P. Kaplan, eds. Academic Press, New York.
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9. O.H. Lowry, N.J. Rosenbrough, A.L. Farr and R.J. Randall (1951) Protein measurement with Folin phenol reagent. J. Biol. Chem. *193,* 265.

8. <u>Annex Picture</u> :

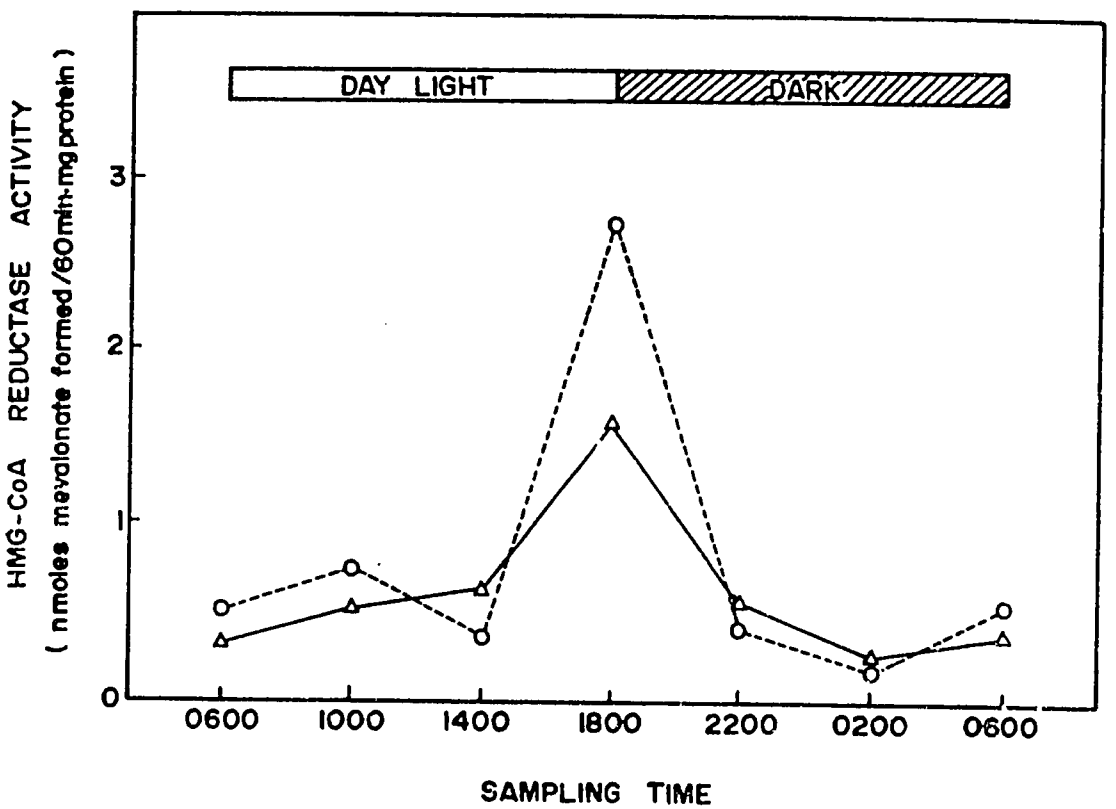

Fig. 1. Diurnal variation of HMGR activity in *Hevea* leaf extracts clones RRIM 600 (O···O) and PB 5/51 (Δ—Δ).

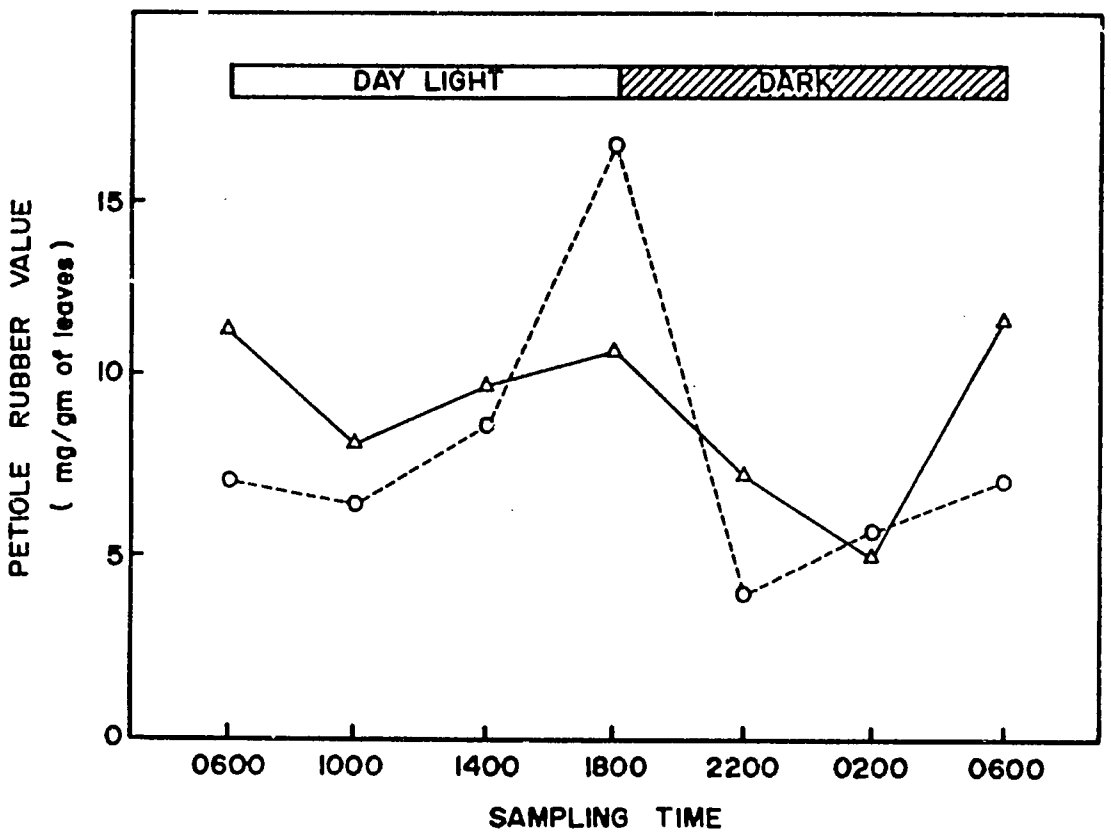

Fig. 2. Diurnal variation of petiole rubber value from *Hevea* leaves of clones RRIM 600 (O---O) and PB 5/51 (Δ——Δ).

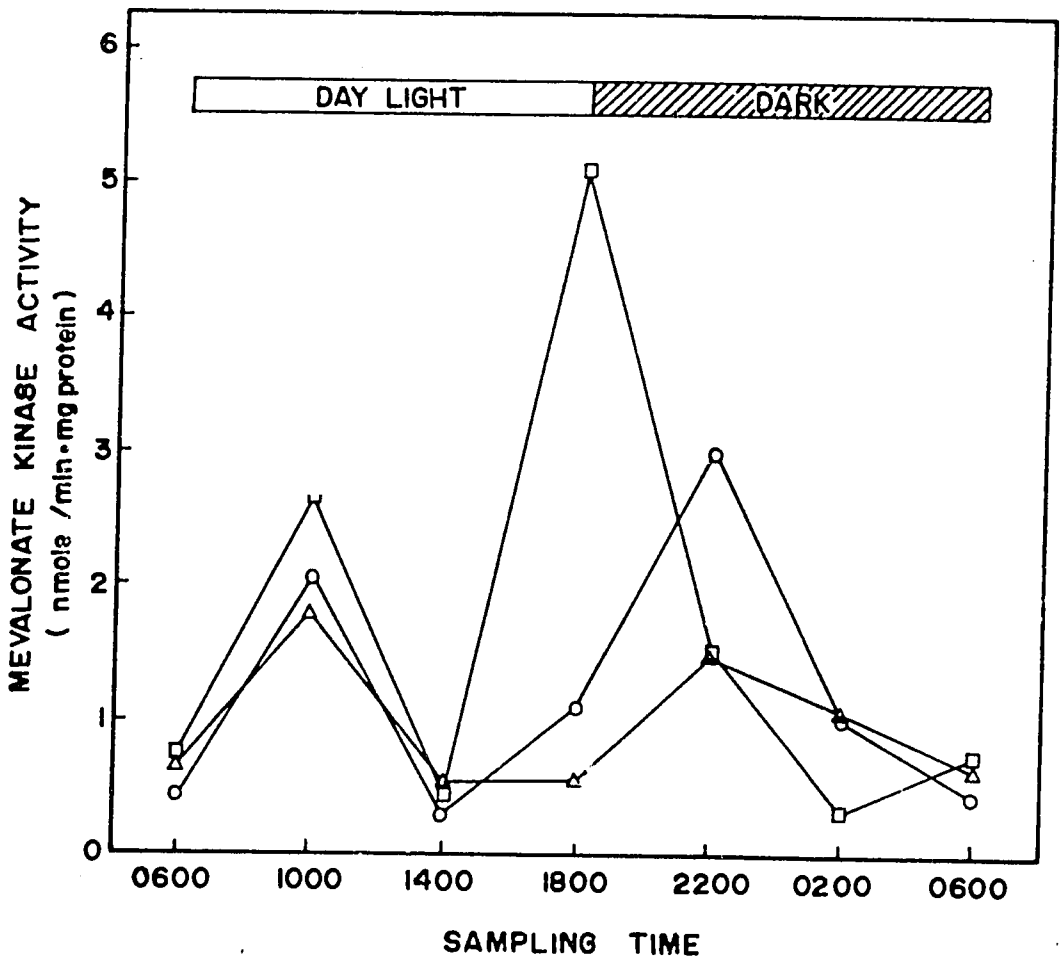

Fig. 3. Diurnal variation of MVA kinase activity in *Hevea* leaf extracts of clones RRIM 600 (O——O), PBM 24 (Δ——Δ) and PB 235 (□——□).

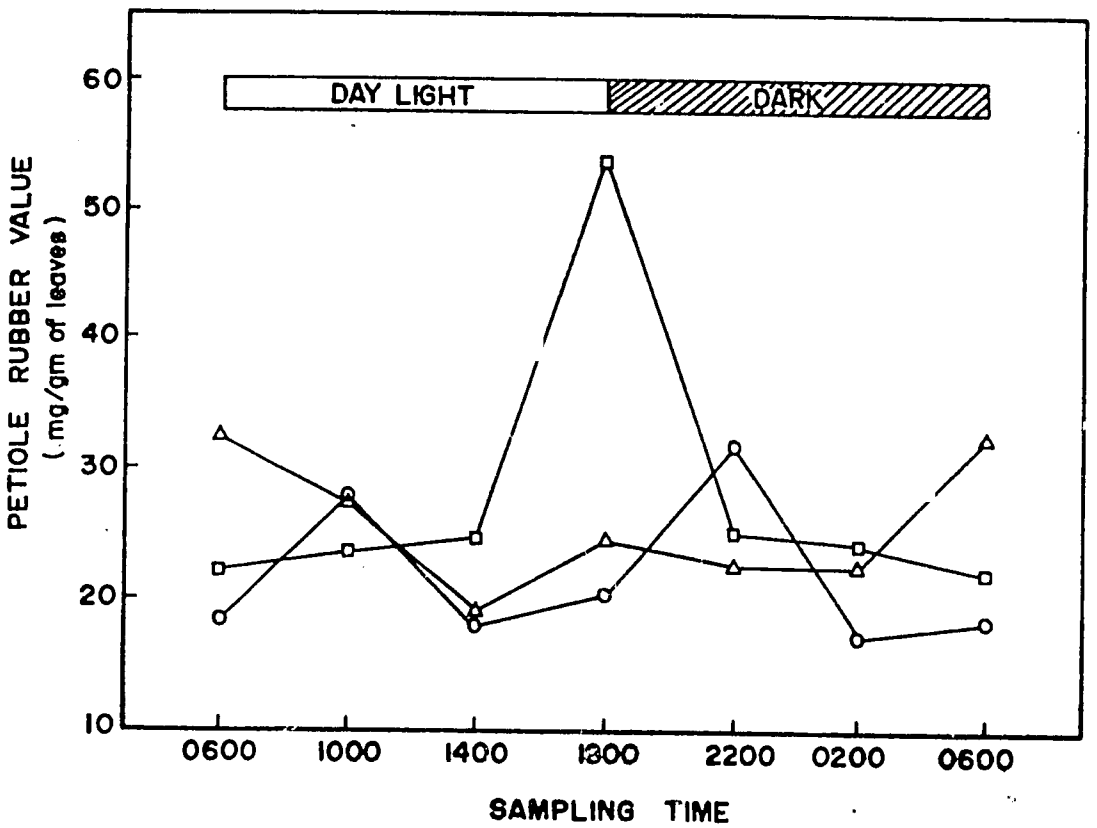

Fig. 4. Diurnal variation of petiole rubber value from *Hevea* leaves of clones RRIM 600 (○——○), BPM 24 (△——△) and PB 235 (□——□).

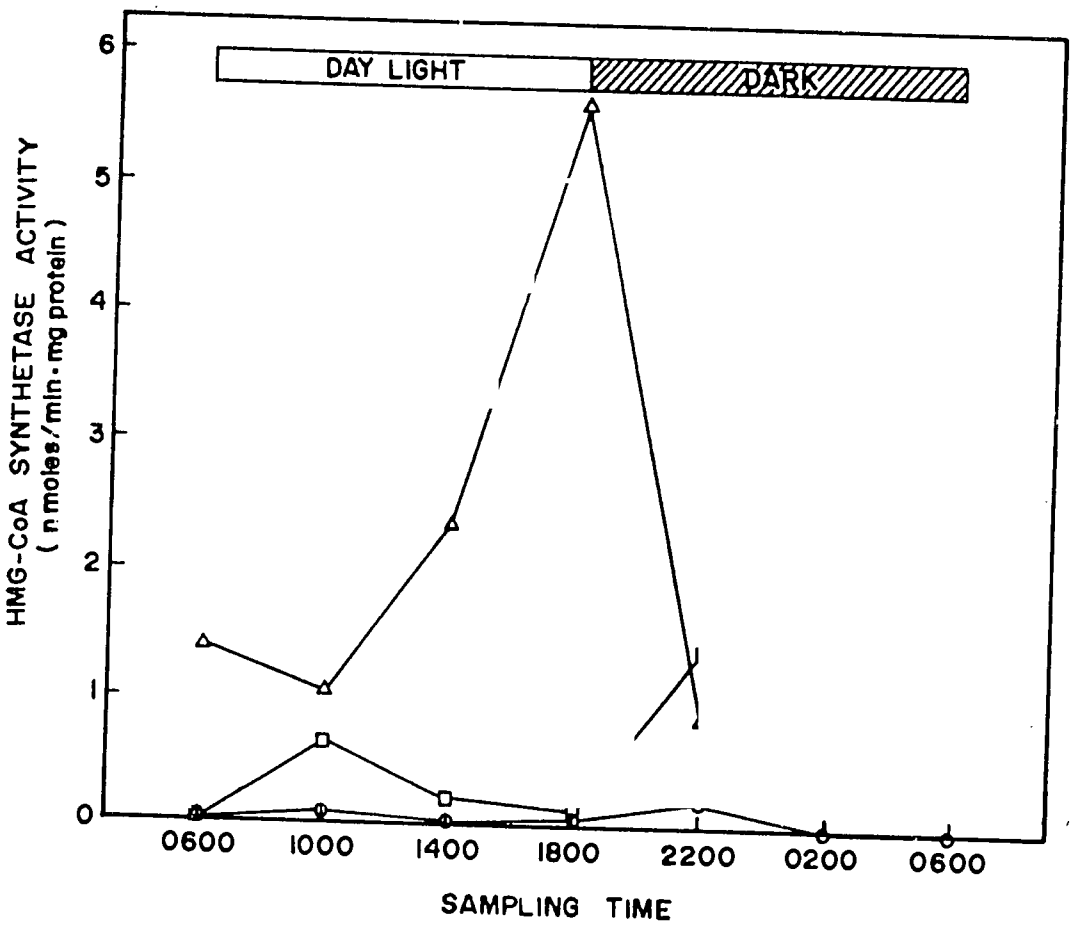

Fig. 5. Diurnal variation of HMG-CoA synthetase activity in *Hevea* leaf extracts of clones RRIM 600 (○—○), BPM 24 (△—△) and PB 235 (□—□).

Table I

Preliminary evaluation for rubber yield on new *Hevea* germplasms

Sample no.	Material source	Clonal code	Yield index*
**1	MT/I/21	24	A
2	MT/I/37A	55	D
3	MT/I/39A	38	B
4	MT/I/39B	39	B+
**5	MT/I/40A	40	A
6	AO/I/10	54	B
7	RO/I/22	60	-
8	RO/I/24	61	B
9	RO/I/44	72	-
10	RO/I/50	76	C
**11	RO/I/54	79	A
12	RO/I/56	81	C
13	RO/I/60	84	D
14	RO/I/56	87	B
15	RO/I/70	91	B
16	RO/I/81	96	B
17	RO/I/105	109	B
18	RO/I/106	110	-
**19	RO/I/109	112	A
20	MT/I/12	15	B
21	RO/I/75	94	-
22	AC/T/2	4/85	B
23	AC/T/3	6/46	B

Table I (continue)

Sample no.	Material source	Clonal code	Yield index*
24	MT/C/1	18/37	B
**25	RO/OP/4	20/66	A
26	RO/A/7	25/3/6	B
27	RO/A/7	25/3/7	C
28	RO/A/7	25/323	B
29	RO/A/7	25/324	D
**30	RO/A/7	25/328	A
31	RO/A/7	25/338	D
32	RO/A/7	25/339	C
33	RO/A/7	25/341	D
34	RO/A/7	25/342	C
35	RO/A/7	25/344	D
36	RO/A/7	25/349	B
37	RO/A/7	25/350	D
38	RO/A/7	25/355	B
39	RO/A/7	25/356	A
40	RO/A/7	25/359	D
41	RO/A/7	25/362	C
42	RO/A/7	25/367	B
43	RO/A/7	25/370	C
44	RO/A/7	25/372	C
45	RO/A/7	25/386	B
46	RO/A/7	25/388	B
47	RO/A/7	25/390	C
48	RO/A/7	25/391	D

Table I (continue)

Sample nc	Material source	Clonal code	Yield index*
49	RO/A/7	25/394	D
50	RO/A/7	25/395	D
51	RO/A/7	25/396	B
52	RO/A/7	25/398	B
53	RO/A/7	25/399	C
54	RO/A/7	25/401	D
55	RO/A/7	25/402	D
56	RO/A/7	25/403	C
57	RO/A/7	25/404	B
58	RO/A/7	25/405	B
59	RO/A/7	25/407	D
60	RO/A/7	25/409	D
61	RO/A/7	25/411	B
62	RO/A/7	25/412	B
63	RO/A/7	25/413	A
64.	RO/A/7	25/415	C
65	RO/A/7	25/418	D
66	RO/A/7	25/420	C
67	RO/A/7	25/421	A
68	RO/A/7	25/423	B
69	RO/A/7	25/424	D
70	RO/A/7	25/425	D
71	RO/A/7	25/427	B
72	RO/A/7	25/430	C
73	RO/A/7	25/432	B

Table I (continue)

Sample no	Material source	Clonal code	Yield index*
74	RO/A/7	25/433	B
75	RO/A/7	25/434	B
76	RO/A/7	25/440	D
77	RO/A/7	25/447	D
78	RO/A/7	25/448	D
79	RO/A/7	25/451	B
80	RO/A/7	25/452	B+
81	RO/A/7	25/455	B
** 82	RO/C/8	24/94	A
83	RO/C/8	24/152	B
84	RO/C/8	24/159	D
85	RO/C/8	24/173	C
86	RO/C/8	24/182	D
87	RO/C/8	24/217	B
88	RO/C/8	24/316	B
89	RO/C/8	24/329	B
90	RO/C/8	24/340	D
91	RO/C/8	24/341	B
** 92	RO/C/8	24/342	A
93	RO/C/8	24/344	B
94	RO/C/8	24/345	B
95	RO/C/8	24/358	C
96	RO/C/8	24/361	B
97	RO/C/8	24/366	C
98	RO/C/8	24/374	D

Table I (continue)

Sample no.	Material source	Clonal code	Yield index*
99	RO/C/8	24/375	D
100	RO/C/8	24/376	D
101	RO/C/8	24/382	D
102	RO/C/8	24/403	B
103	RO/C/8	24/409	D
104	RO/C/8	24/411	B
105	RO/C/8	24/412	D
106	RO/C/8	24/416	C
107	RO/C/8	24/423	D
108	RO/C/8	24/425	D
109	RO/C/8	24/435	D
110	RO/C/8	24/437	C
111	RO/C/8	24/439	B
112	RO/C/8	24/446	B
113	RO/C/8	24/453	B
114	RO/C/8	24/454	B
115	RO/C/8	24/457	C
116	RO/C/8	24/458	C
117	RO/C/8	24/459	C
118	MT/IT/12	26/1	C
119	MT/IT/12	26/7	C
120	MT/IT/12	26/9	C
121	MT/IT/12	26/12	C
122	MT/IT/12	26/14	B
123	MT/IT/12	26/26	C

Table I (continue)

Sample no.	Material source	Clonal code	Yield index*
124	MT/TT/12	26/32	B
125	MT/IT/12	26/37	B
126	MT/IT/12	26/45	C
127	MT/IT/12	26/49	B
128	MT/IT/12	26/55	D
129	MT/IT/12	26/64	C
130	MT/IT/12	26/67	C
131	MT/IT/12	26/70	D
132	MT/IT/12	26/77	B
133	MT/IT/12	26/79	B
134	MT/IT/12	26/81	C
135	MT/IT/12	26/84	C
136	MT/IT/12	26/86	B
137	MT/IT/12	26/122	B
138	MT/IT/12	26/128	C
139	MT/IT/12	26/130	B
140	MT/IT/12	26/133	B
141	RO/A/7	25/1	B
142	RO/A/7	25/5	C
143	RO/A/7	25/8	D
144	RO/A/7	25/14	C
145	RO/A/7	25/44	D
146	RO/A/7	25/45	D
147	RO/A/7	25/52	C
148	RO/A/7	25/96	D

Table I (continue)

Sample no.	Material source	Clonal code	Yield index*
149	RO/A/7	25/103	D
150	RO/A/7	25/111	D
151	RO/A/7	25/122	C
152	RO/A/7	25/127	C
153	RO/A/7	25/163	C
154	RO/A/7	25/166	D
155	RO/A/7	25/174	C
156	RO/A/7	25/189	C
157	RO/A/7	25/190	D
158	RO/A/7	25/197	D
159	RO/A/7	25/225	C
160	RO/A/7	25/239	C
161	RO/A/7	25/242	D
162	RO/A/7	25/249	B
163	RO/A/7	25/250	B
164	RO/A/7	25/254	D
165	RO/A/7	25/265	C
166	RO/A/7	25/269	B
167	RO/A/7	25/279	D
168	RO/A/7	25/281	C
169	RO/A/7	25/286	D
170	RO/A/7	25/292	C
171	RO/A/7	25/307	B
172	RO/A/7	25/310	C
173	RO/A/7	25/313	D

Table I (continue)

Sample no.	Material source	Clonal code	Yield index*
174	RO/A/7	25/464	B
175	RO/A/7	25/465	D
176	RO/A/7	25/467	D
177	RO/A/7	25/468	C
178	RO/A/7	25/471	C
179	RO/A/7	25/472	D
180	RO/A/7	25/473	D
181	RO/A/7	25/474	D
182	RO/A/7	25/477	D
183	RO/A/7	25/480	D
184	RO/A/7	25/481	D
185	RO/A/7	25/482	C
186	RO/A/7	25/487	C
187	RO/A/7	25/489	D
188	RO/A/7	25/491	D
189	RO/A/7	25/493	C
190	RO/A/7	25/494	C
191	RO/A/7	25/497	C
**192	RO/A/7	25/500	A
193	RO/A/7	25/505	D
194	RO/A/7	25/508	D
195	RO/A/7	25/501	D
196	RO/A/7	25/511	B
197	RO/A/7	25/516	B
**198	RO/A/7	25/519	A

Table I (continue)

Sample no	Material source	Clonal code	Yield index*
199	RO/A/7	25/513	B
**200	RO/A/7	25/539	A
201	RO/A/7	25/545	B
202	RO/A/7	25/554	B
203	RO/A/7	25/576	D
204	RO/A/31	25/577	D
205	RO/A/31	25/578	D
206	RO/A/31	25/579	C
217	RO/A/31	25/580	C
208	RO/A/31	25/583	C
209	RO/A/31	25/585	D
210	RO/A/31	25/590	D
211	RO/A/31	25/592	B
212	RO/A/31	25/605	D
213	RO/A/31	25/608	C
214	RO/A/31	25/609	B
215	RO/A/31	25/610	D
216	RO/A/31	25/593	C
217	RO/A/31	25/595	B
218	RO/A/7	25/593	C
219	RO/A/7	25/595	B
220	RO/A/7	25/596	D
221	RO/A/7	25/603	D
222	RO/A/7	25/604	B
223	RO/A/7	25/611	B

Table I (continue)

Sample no.	Material source	Clonal co	Yield ind
*224	RO/A/7	25/613	A
225	RO/A/7	25/614	C
226	RO/A/7	25/615	C
227	RO/A/7	25/616	C
228	RO/A/7	25/619	B
229	RO/A/7	25/620	B
230	RO/A/7	25/621	C
231	RO/A/7	25/624	D
232	RO/A/7	25/70	B
233	AC/3/8	35/3	C
234	AC/3/8	35/6	C
235	AC/3/8	35/12	C
236	AC/3/8	35/21	D
237	AC/3/8	35/23	C
238	AC/3/8	35/42	C
239	AC/3/8	35/43	D
240	AC/3/8	35/44	D
241	AC/3/8	35/30	D
242	AC/3/8	35/31	C
243	AC/3/8	35/36	D
244	AC/3/8	35/39	D
245	AC/3/8	35/49	D
246	AC/3/8	35/51	C
247	AC/3/8	35/52	
248	AC/3/8	35/53	

Table I (continue)

Sample no.	Material source	Clonal code	Yield code*
249	AC/3/8	35/56	B
250	AC/3/8	35/62	B
251	AC/3/8	35/66	B
252	AC/3/8	35/67	B
253	AC/3/8	35/74	C
254	AC/3/8	35/84	C
255	AC/3/8	35/88	C
256	AC/3/8	35/91	B
**257	AC/3/8	35/105	A
258	AC/3/8	35/114	B
259	AC/3/8	35/120	C
**260	AC/3/8	35/134	A
**261	AC/3/8	35/136	A
262	AC/3/8	35/170	D
263	AC/3/8	35/182	C
264	AC/3/8	35/195	C
265	AC/3/8	35/197	B
266	AC/3/8	35/203	C
267	AC/3/8	35/237	B
268	AC/3/8	35/243	B
269	AC/3/8	35/247	B
270	AC/3/8	35/281	B
271	AC/3/8	35/312	B
*272	AC/3/8	35/364	A
273	AC/3/8	35/381	C

Table I (continue)

Sample no.	Material source	Clonal code	Yield index*
274	AC/3/8	35/390	C
275	AC/3/8	35/404	C
276	AC/3/8	35/413	C
277	AC/3/8	35/415	D
278	AC/3/8	35/416	B
279	AC/3/8	35/418	A
300	AC/3/8	35/425	D
301	AC/3/8	35/431	B
302	AC/3/8	35/434	D
303	AC/3/8	35/436	D
304	AC/3/8	35/444	B
305	AC/3/8	35/445	D
306	AC/3/8	35/453	D
307	AC/3/8	35/445	D
308	AC/3/8	35/453	D
309	AC/3/8	35/458	D
310	AC/3/8	35/460	B
311	AC/3/8	35/467	D
**312	AC/3/8	35/470	A
313	AC/3/8	35/475	C
314	AC/3/8	35/479	B
315	AC/3/8	35/497	C
**316	AC/3/8	35/506	A
**317	AC/3/8	35/518	A
318	AC/3/8	35/549	B

Table I (continue)

Sample no.	Material source	Clonal cod	Yield index*
319	AC/3/8	35/551	D
320	AC/3/8	35/552	
321	AC/3/8	35/553	
322	AC/3/8	5/8	
**323	RO/A/7	25/424	
324	MT/IT/12	26/32	
325	AC/T/1	5/68	
326	AC/T/1	5/88	
327	AC/T/1	5/90	
328	AC/T/1	5/107	
329	AC/T/1	5/128	
**330	AC/T/1	5/140	[illegible]
331	AC/T/1	5/166	I
332	AC/T/1	5/168	C
333	AC/T/3	6/35	I
334	AC/T/3	6/36	E
335	AC/T/3	6/46	C
336	MT/C/4	7/15	E
337	MT/C/4	7/37	B
338	MT/C/4	7/55	B
339	MT/C/4	7/113	B
**340	MT/C/4	7/135	A
341	MT/C/4	7/159	C
342	MT/C/7	8/25	B
343	MT/C/2	10/4	B

Table I (continue)

Sample no	Material source	Clonal cod	Yield index*
344	MT/C/2	10/10	C
345	MT/C/2	10/15	D
346	MT/C/2	10/17	B
347	MT/C/2	10/26	B
**348	MT/C/2	10/27	A
**349	MT/C/2	10/47	A
350	RO/PB/2	3/2/9	C
351	RO/PB/2	3/223	B
352	RO/PB/2	3/283	D
**353	RO/PB/2	3/287	A
354	RO/PB/2	3/3/4	B
**355	RO/PB/2	3/330	A
356	RO/PB/2	3/352	C
357	RO/PB/2	3/367	C
358	RO/PB/2	3/370	D
359	RO/PB/2	3/396	B
360	RO/PB/2	3/450	B
361	RO/PB/2	3/467	D
362	RO/PB/2	3/504	B
363	AC/T/1	4/15	D
364	AC/T/2	4/19	C
365	AC/T/1	5/4	B
366	AC/T/1	5/8	C
367	AC/T/1	5/10	D
368	AC/T/1	5/41	C

Table I (continue)

Sample no.	Material source	Clonal code	Yield index*
369	AC/T/1	5/44	D
370	AC/T/1	5/62	D

*Yield index was graded by evaluating from the amount of latex being excreted from rubber leaflet upon cutting through leaf blade parallel to midrib vien. Grades A to D were given for high to low latex yield obtained.

**Selected for further yield screening by HMGR assay (see Table II).

Table II

Studies on latex HMGR activity from new *Hevea* germplasms

Material sour	Clonal code	HMGR activity*
Wickham	RRIM 600	3.87
MT/I/21	24	8.94
MT/I/40A	40	1.34
RO/I/54	79	2.68
RO/OP/4	20/66	1.61
RO/A/7	25/328	1.49
RO/A/7	25/356	1.30
RO/A/7	25/413	2.07
RO/A/7	25/421	1.56
RO/A/7	24/94	2.11
RO/A/7	24/342	1.31
RO/A/7	25/500	10.87
RO/A/7	25/519	3.89
RO/A/7	25/539	6.79
RO/A/7	25/611	2.56
AC/3/8	35/91	1.74
AC/3/8	35/134	2.30
AC/3/8	35/364	2.45
AC/3/8	35/416	2.34
AC/3/8	35/470	1.94
AC/3/8	35/518	7.88
RO/A/7	25/424	

Table II (continue)

Material source	Clonal code	HMGR activity*
AC/T/1	5/140	2.08
MT/C/4	7/135	5.02
MT/C/2	10/27	23.63
MT/C/2	10/47	3.32
RO/PB/2	3/287	9.23

*HMGR activity is expressed per ml of latex and equivalent to nmole of MVA formed per min.