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PROGRESS REPORT NO. 3

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

A RESEARCH PROJECT

USAID/PSTC PROGRAM

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Submitted by

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Project Profile

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Project Title	: Biotechnology Application for Characterization and Selection of Better-Yielding Rubber Clones.
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1. Background/Introductions :

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

Traditional practice on yield evaluation of rubber trees 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 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.

Yield screening at seedling stage (Part A)

Potential low yielder (discard) | Potential high yielder

Disease resistance screening at tissue culture stage (Part B)

Susceptible clone | resistant clone

Adopted as possible high yielding disease resistant clones.

3. 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, polyoxyethylene ether W-1 (Brij W-1), mevalonolatctone, PEP, NADH, pyruvate kinase, lactate dehydrogenase, acetyl-CoA, acetoactyl-CoA, polyvinyl pyrrolidone were from Sigma. 3-^{14}C HMG-CoA was from Amersham England and Affi-Gel-Blue was from Bio-Rad.

Collection and fractionation of latex :

Latex was obtained from several clones of *Hevea brasiliensis* as indicated in the text. The trees are regularly tapped, half spirally on every other day (S.2/D.2). All clones are grown categorized at the Rubber Research Center, Hat-Yai. Rubber trees were normally tapped at 0600 h or otherwise as indicated. After tapping the fresh latex was collected into an ice-chilled beaker. The latex from each clone was pooled and fractionated into 3 major fractions by centrifugation in a microfuge at 15,000 rpm for 60 min. They are the rubber fraction, the C-serum (latex cytoplasm) and the bottom fractions. respectively. The bottom fractions, where 95% of HMGR was located (4), were separated by cutting the bottom of the microtube and used for assay of HMGR activity. The C-serum was used for assays of mevalonate kinase and HMG-CoA synthetase activities.

Assay of HMG-CoA reductase (HMGR) activity :

The HMGR activity was a ssayed in buffer X (0.1 M KCl, 10 mM DTT 30 mM EDTA. 0.1 M K_xPO_4.pH 7.0) containing bottom fraction or active protein fraction or fresh latex with protein ranged from 0.1-1.8 mg, 0.4 µmoles NADPH, 120 nmoles DL-3 ^{14}C HMG-CoA (1000 dpm/nmole) in a final volume of 50-100 µl. For solubilized enzyme, 1% W/V of BSA was added to the assay system to stabilize the HMGP activity. Incubation was at 37°C for 20 or 30 min as indicated The reaction was stopped by addition of 25 µl of 10 N HCl. Unlabeled mevalonolactone was then added, and the mixture was allowed to incubate for another 30 min for completelactonization 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 mevolonate (MVA) kinase activity :

MVA kinase activity in c-serum was assayed by measuring the amount of ADP released from the reaction. 0.1 ml of C-serum was incubated with 5 µmoles ATP and 3 µmoles potassium mevalonate for 10 min at 30°C. The reaction was carried out in 50 mM phosphate buffer. pH 7.0, 5 mM $MgCl_2$, and 40 mM KCl in a final volume of 0.5 ml as modified from the method of Gray *et al.* (6). Mevalonate was omitted in the controls. The reaction was stopped by adding 0.2 ml of 1 M trichloroacetic acid and the precipitate was separated by

centrifugation at 10,000 rpm for 3 min. The clear supernatant was neutralized with 0.1 ml of 2 M KOH. The aliquots (0.1-0.4 ml) were then used for ADP determination using coupled enzyme catalyzed oxidation of NADH. The ADP assay mixture contained 4 µmoles phosphoenol pyruvate (PEP), 0.3 µmoles NADH with pyruvate kinase (7 µg protein, sp.act. 477 units/mg) and lactate dehydrogenase (39 µg protein sp.act. 490 units/mg). The assay was carried out in 50 mM potassium phosphate buffer pH 7.0 containing 5 mM $MgCl_2$ and 40 mM KCl in a total volume of 1.0 ml. MVA kinase activities in the C-serum were determined either immediately after collecting or kept in the cold assayed in the morning for samples collected during the night. All determinations were done within 15 hours after latex collection.

Assay of HMG-CoA synthetase activity :

HMG-CoA synthetase activity was determined using dialyzed C-serum. The assay 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 contained 1 M Tris HCl pH 8.2, 0.1 mM EDTA, 0.2 mM 1-^{14}C acetyl-CoA (4×10^5 cpm/µmole), 0.05 mM acetoacetyl-CoA, and dialyed C-serum (0.5-2 mg protein/ml) in a final volume of 0.2 ml. (7). 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 µl aliquots aliquots are transferred into glass vials containing 0.1 ml 6 M HCl. The acidified aliquots were taken to dryness at 95°C using heating plate. Water (0.5 ml) is added to dissolved 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 using controls in which C-serum was omitted.

Part B. Disease resistance screening of Hevea at tissue culture stage

Explant materials

Hevea flowers were obtained from late flowering season. Pistillate flowers located at the top of central axis and main lateral branches were collected. Male flower buds (3-3.5 mm) in which majority of the anthers are at uninucleate stage were excised and used for callusing in various types of culture media as indicated.

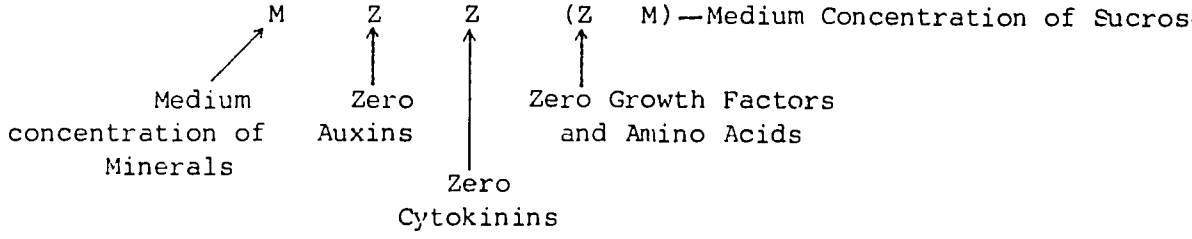
Medium preparation for *Heave* tissue culture

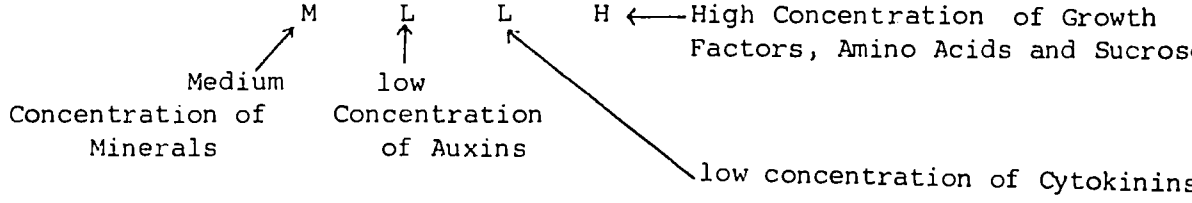
1. MB medium

macronutrients		
KNO_3	954	mg/l
NH_4NO_3	650	mg/l
$MgSO_4.2H_2O$	370	mg/l
$CaCl_2$	530	mg/l
KH_2PO_4	503	mg/l
micronutrients		
H_3BO_3	1747	mg/l
$MnSO_4.4H_2O$	1003	mg/l
$ZnSO_4.7H_2O$	8325	mg/l
$Na_2MoO_4.5H_2O$	241.9	mg/l
$CuSO_4.5H_2O$	249	mg/l
Iron Salts		
Na_2-EDTA	40.9	mg/l
$FeSO_4.7H_2O$	30.5	mg/l
Organic gr. Substance		
Nicotinic acid	492.4	mg/l
Glycine	2252.1	mg/l

Thiamine	505.9	mg/l
Myo-inositol	100.8	mg/l
Pyridoxine	493.5	mg/l
Sucrose	69829.2	mg/l
Water coconut	4%	
Hormone		
Kinetin	4.6	µM
2,4-D	4.5	µM
NAA	5.7	µM
pH	5.8	
Agar	1.3%	

2. MZZ (ZM) and MLLH media





L = Low concentration

M = Medium concentration

H = High concentration

Z = Zero concentration

Concentrations of Mineral

Macronutrients (mM)	Low	Medium	High
NH_4NO_3	2	10	20
KNO_3	2	10	20
NaH_2PO_4	0.2	1	2
$CaCl_2$	0.3	1.5	3
$MgSO_4$	0.3	1.5	3

Micronutrients (mM)	Low	Medium	High
H_3BO_4	1.5	75	150
$MnSO_4$	10	50	100
$ZnSO_4$	4	20	40
$CuSO_4$	0.01	0.05	0.1
Na_2MoO_4	0.1	0.5	1
$CoCl_2$	0.01	0.05	0.1
KI	0.5	2.5	5
FeNaEDTA	10	50	100

Concentrations of growth factors

Growth factors (µM)	Low	Medium	High
inositol	60	300	600
nicotinic acid	4	20	40
pyridoxine HCl	0.6	3	6
thiamine HCl	4	20	40
Biotin	0.1	0.5	1
D-Ca-pantothen	0.5	2.5	5
riboflavin	1	5	10
ascorbic acid	1	5	10
choline chloride	1	5	10
Amino acid (µM)			
L-cysteine HCl	12	60	120
Glycine	5	25	50
Concentration of auxin (µM)			
IAA	0.1	1	10
IBA	0.1	1	10
NAA	0.1	1	10
2,4-D	0.1	1	10
Concentrations of cytokinin (µM)			
kinetin	0.1	1	10
BAP	0.1	1	10
Sucrose (mM)	12	60	120
pH 5.5			
Agar 1.3%			

4. Results/Dicussions/Tables :

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

A.1 Interclonal and intraclonal correlations between the whole latex HMGR activities and rubber yields.

The reported results discussed in progress report no. 2 showed good interclonal correlation between the total HMGR activities and rubber yields (see Table 4). Results obtained from 10 different clonal types selected for the study showed highly positive correlations. The correlation between HMGR activity and rubber yield among 10 different clones as depicted in Fig. 1. was calculated and shown to be of highly statistical significance with values of $\alpha = 0.01$ and $r = 0.78$. In addition, the study was extended to investigate the intraclonal correlation Ten different trees of clone RRIM 600 were investigated for correlation between the whole latex HMGR activities and rubber yields. Good positive correlation ($\alpha = 0.01, r = 0.76$) was also obtained in this intraclonal study as shown in Fig. 2. These studies clearly indicated the high degree of positive correlations. Both interclonal and intraclonal correlations between HMGR activities and rubber yields suggested a possible direct and important role of the HMGR enzyme in controlling of rubber biosynthesis. Furthermore, the results help substantiate the possible use of HMGR as a useful and practical biochemical parameter for early selection of potential high yielding clones. The selection of superior clones can be done early at seedling stage to prevent the planting of poor yielders. This approach can save several years in time as compared to the present evaluation practice.

If the correlations can be shown to remain consistent and reliable from seedling through the tapping period, the early selection of potential high-yielding clones with HMGR could possibly become a new innovative practice and in common use in the near future.

A.2 Correlation between HMGR activities and rubber yields in trees rated as high. medium, and low yielding rubber trees.

Variation of rubber productivity within individual *Hevea* clonal type (intraclonal variation) was shown and discussed previously in the progress report no. 2. In this study, we have identified the high, medium, and low yielding rubber trees for correlation studies. The trees from two colonal types, RRIM 600 and KRS 208, were used in the experiments. Randomly selected rubber trees (9-10 trees) from each clone were tapped and measured for rubber yields. Results of the average rubber yield from 5 tappings per tree were shown in Fig. 3 (clone RRIM 600) and Fig. 4 (clone KRS 208). The tree number 8,4, and 7 from clone RRIM 600 were rated and selected to represent low, medium, and high yielding trees respectively (see Fig. 3) for this study. For clone KRS 208, the tree number 3,5, and 6 were rated as low, medium and high yielding trees respectively (see Fig. 4). The correlation studies were carried out between rubber yield and total HMGR activity as well as HMGR activity per ml latex. The activity of HMGR in latex collected for 5 minutes after the 15 min tapping (between 15th-20th min whence relatively constant HMGR level occurs, see report no.1) was also measured and compared. The results of these studies are shown in Table 1 for clone RRIM 600 and in Table 2 for clone KRS 208. Highly positive correlations were seen for both clones. When the rankings of rubber yields and HMGR activities were compared, they showed highly correspondent ranking positions. The high-yielders and

low-yielders have highest and lowest total HMGR activities accordingly. The same correspondent correlations were also observed with HMGR activity per ml. latex. The statistical significance of these studies were ratified and ascertained with calculated r values of 0.85 0.99 as indicated in the Tables. These findings provide supportive evidence for the possible direct and important role of HMGR in controlling rubber biosynthetic capacity in rubber trees as similarly suggested from other results.

A.3 Preliminary yield screening on new *Hevea* germplasm

Attempt on preliminary yield screening on 160 new *Hevea* germplasms were made on *Hevea* leaf latex. This was carried out by evaluating the yield on rubber latex upon cutting through leaflet of 3 months old seedlings using method of Zhongyu *et al.* (8). Leaf blade was cut parallel on both sides of midrib vein with 1 cm apart. Amount of latex being excreted from lateral veins were then measured. Grades A to E were given for high to low latex yield obtained. Results on yield evaluation by estimating latex excreted from lateral veins are shown in Table 3. There were only 10 out of 160 new *Hevea* germplasms which demonstrate high yield potential. They are subjected to further yield evaluations by comparing their latex HMGR level obtained by making microtapping on the stem and compared with RRIM 600 latex under the same condition and treatment. Results on latex HMGR levels on RRIM 600 and the 10 new *Hevea* germplasms are shown on Table 4. Most of the potential high yielders selected from 160 new *Hevea* germplasms have lower latex HMGR activity when compared to RRIM 600 which is the established known high yielder. There is only one new clonal type from MT/C/1 type of material source under 18/5 coding that demostrated higher latex HMGR activity than the RRIM 600 clone. Presently we obtained more than ten thousounds of new *Hevea* germplasms, it

is therefore likely to obtain more high yield potential clone by using similar screening technique. More extrnsive screening is underway and comparison can be made more meaningful when the data are pooled for statistical analysis.

A.4 Correlation studies on latex MVA kinase & HMG-CoA synthetase activity levels and rubber yield in high, medium and low yielding rubber trees.

High, medium and low yielding rubber trees clone RRIM 600 were identified as previously described. It is seen on table 5 that levels of MVA kinase and HMG-CoA synthetase activities have negative correlation with rubber yield. High yielding trees contain low level of MVA kinase and HMG-CoA synthetase. Low yielding trees contain high and intermediate levels of MVA kinase and HMG-CoA synthetase. Since positive correlations between rubber yield and these two enzymes (MVA kinase & HMG-CoA synthetase) cannot be established, the regulatary role of the two enzymes seemed to be of less importance. It is therefore unlikely for MVA kinase and HMG-CoA synthetase to have direct roles in regulation of rubber biosynthesis.

A.5 Latex HMG CoA synthetase activity and its diurnal variation

Diurnal varition of HMG CoA synthetase was investigated in latex collected from rubber clones, RRIM 600, GT 1 and KRS 21 Fig. 5 showed the variation of HMG-CoA synthetase activities in C serum obtained at different time during the 24 hour cycle. Each point was the average of two values obtained from seperate tappings, 14 days apart. The HMG-CoA synthetase activity of each clone tend to vary during 24 hour cycle. The different between high and low activity values are less dramatic as compared to those found in HMGR and MVA kinase (progress report no. 2). High and low activity values were observed at 0200 and 1800 respectively. Data on diurnal

variation of dry rubber content of the same latex samples used for HMG-CoA synthetase assay were shown in Fig. 6. High and low rubber contents were observed between 1400-1800 h and 0200-0600 h, respectively. The high and low level of HMG-CoA synthetase are not correspond to those of dry rubber content in this study. Inverse correlation was found at 1800 h where HMG-CoA synthetase was highest and dry rubber content was lowest.

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

B.1 Callus culture from *Hevea* male flowers

Hevea anthers were used for callus generation in the study. We were able to generate callus from male flowers of clones PB 310. RRIC 6 and GT 1 (Table 6) in additions to clones RRIM 600, PR 255 as previously reported. Maximum size were obtained from RRIM600 callus after 12 weeks period with a diameter of 3 mm. Most callus in this study began to turn brownish from yellowish-green colour and died following the 4th week. Callus from RRIC 6 clone appeared to be capable of growing faster than those from GT 1, PB 310 and RRIM 600 in the same MB medium under the study condition.

We were not able yet to maintain our callus culture for further study on disease resistance by subculturing into MB medium or modified MS medium as described by chen *et al.* (9) for *Hevea* anther culture. Several attempts are underway for successful subculturing of the calli. This failure may result from the use of unsuitable media. We, therefore, plan to search for a suitible media by applying broad spectrum media described by De Fossard (10).

During the second flowering season (Aug-Oct) we were able to obtain only male flowers from RRIC 6 clone in large enough quantity to start exploring the broad spectrum culture media. Preliminary results as shown in Table 7 revealed slower rate of callus growth in MLLH media as compared to the MB media. A total number of 18 combinations between high, medium and low concentrations of minerals, auxin, cytokinin, growth factors, amino acids and sucrose will be tested in order to find a suitable media for cullus maintenance.

B.2 *Hevea* Callus histoligical studies :

Callus tissues were fixed in glutaraldehyde (1% in phosphate buffer pH 7) at 4°C, dehydrated through an alcohol series (30,50,70,80 and 95% absolute ethanol) and embedded in paraplast. Sectioning was made by rotary microtome (8 μ). The cross-sections were affixed to slide using Hupt's adhesive, dewaxed in xylene, dehydrated with alcohol series, stained in 1% toluidene blue for 2-5 minutes and washed out the excess dye with water. The sections were then dehydrated with alcohol series and mounted in eurapol. Photagraphs were taken under Nikon inverted microscope. As shown in Fig. 8, mass of callus cells seen as phenol-blackened spots (Fig. 7) were stained as redish purple while normal cells were greenish blue. The arrangement of cells appeared to be non-embryogenic like structure.

B.3 Lesion studies with live pathogen infection on *Hevea* leaves :

Several young leaves from RRIM 600 and GT 1 were detached from young branches. The leaves were washed several time with distilled water and incubated in moist chambers. Small lesions on leaf surface were made and placed with small drop of phytopthora mycelium suspension. After 3-4 days,

infection signs were seen at the fungal application sites in all leaf samples. From this study, both susceptible (RRIM 600 clone) and resistant (GT 1 clone) leaves were infected upon mycelium application through leaf injuries. It is not certained whether the leaf lesion treatment used for this study is too severs to cause false positive results on GT 1 clone. We are planing for milder treatment on leaf samples by application of spore samples suspended in Tween 20 on leaf surface without making any prior lesions. Better assesments on live pathogen infection on *Hevea* leaves can be made after the later study.

5. Conclusion remarks :

- Good positive interclonal and intraclonal correlations between the whole latex HMGR activities and rubber yields were obtained.
- A new clonal type from MT/C/1 type of material source under coding 18/5 was indentified as potential high yielder from a preliminary yield screening on 160 new *Hevea* germplasms
- There was no direct correlation between latex MVA kinase & HMG-CoA synthetase activity levels and rubber yield in high, medium and low yielding rubber trees.
- Study on diurnal varitation of HMG-CoA synthetase and dry rubber yield showed negative correlations between high and low activity levels of HMG-CoA synthetase and dry rubber contents.
- Callus could be initiated from male flowers of clones PB 310, RRIC 6 and GT 1.
- Histological studies on *Hevea* callus revealed many phenol-blankened spots on dying tissue.

6. Work plan for next period :

- Diurnal variation of MVA kinase, HMG-CoA synthetase in *Hevea* leaves will be studied.
- Immunological studies on purified HMGR is planned for the next period.
- Further attempts will be carried out for a successful subculturing of *Hevea* callus.
- Toxin from suspension culture of *phytopthora botryosa* will be isolated and used for the disease resistance study.

7. Reference :

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8. Annex Picture :

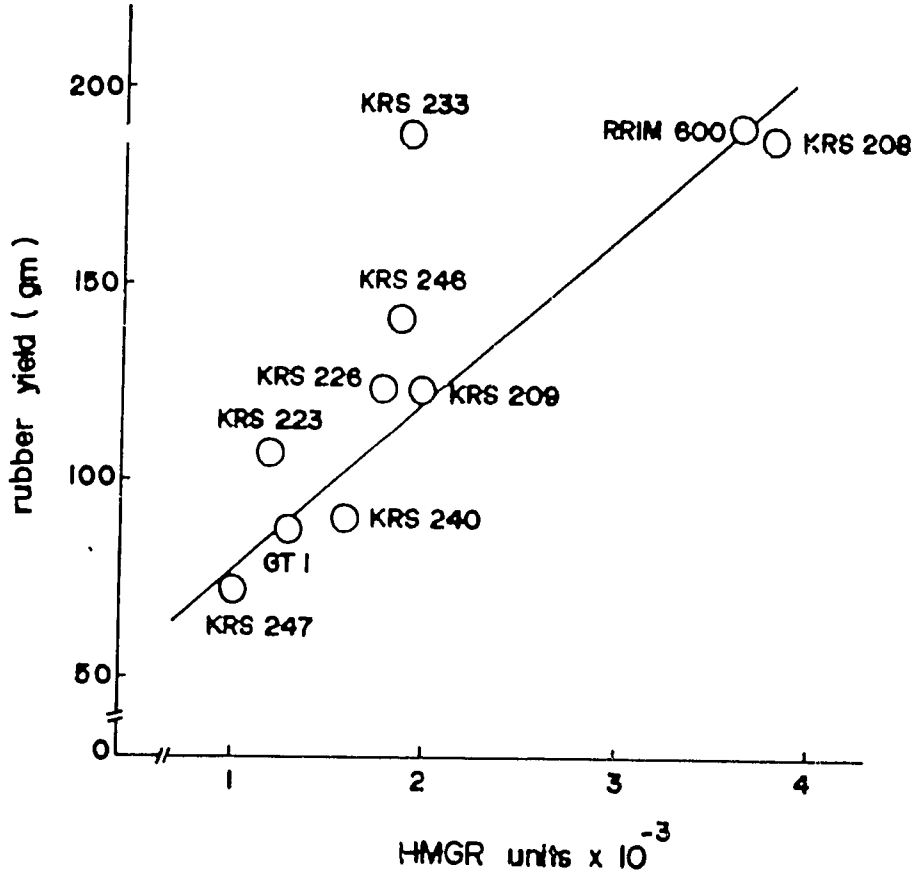

Fig.1. Interclonal correlation between whole rubber yield and HMGR activity in ten clones. Each value is the mean of 3 tappings x 3 trees per clone. The correlation is highly significant (α = 0.01, r = 0.78). The unit of enzyme activity was defined as nmoles mevalonate formed per minute.

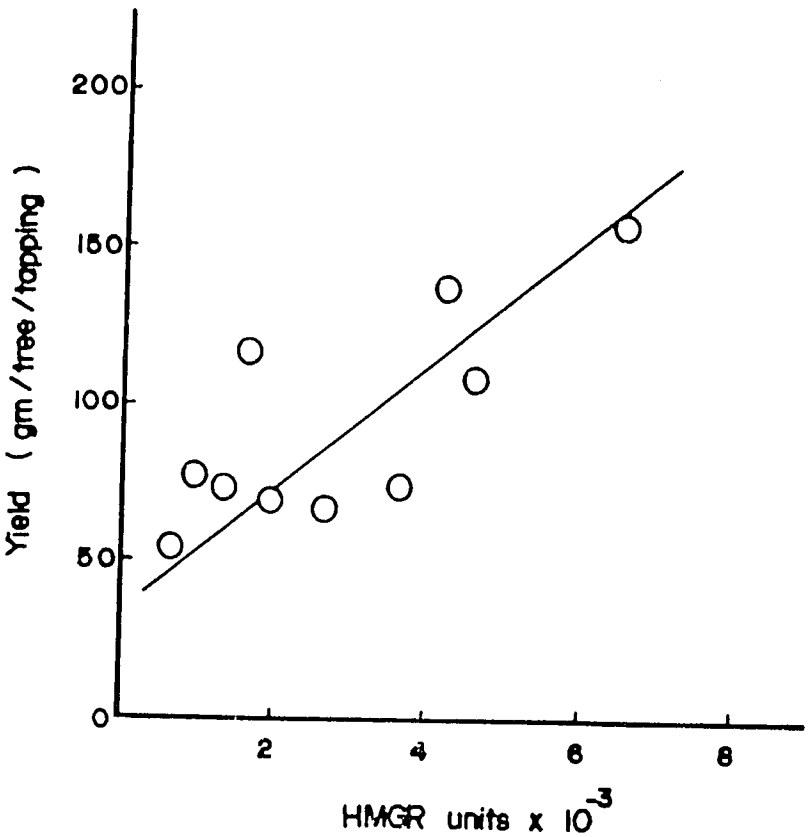

Fig.2. Intraclonal correlation between total rubber yield and HMGR activity in 10 RRIM 600 trees. Each value is the mean of 2 tappings. The correlation is highly significant ($\alpha = 0.01$, $r = 0.76$). The unit of enzyme activity was defined as nmoles mevalonate formed per minute.

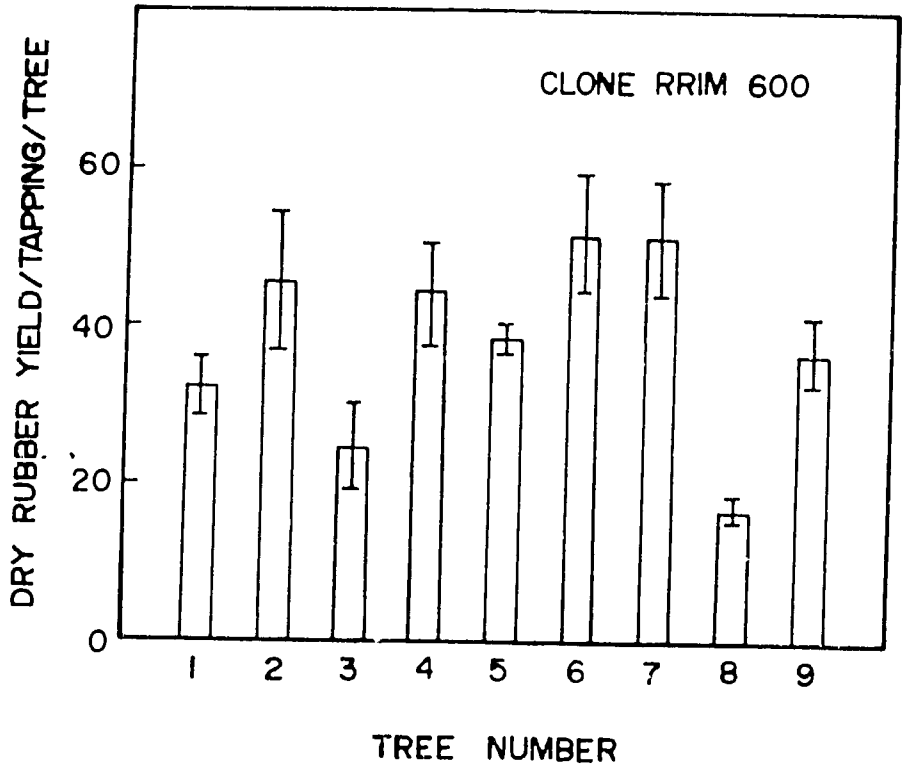

Fig.3. Intraclonal variation of rubber yield per tapping per RRIM 600 tree. Each value is the mean ± SD of 5 tapping measurements.

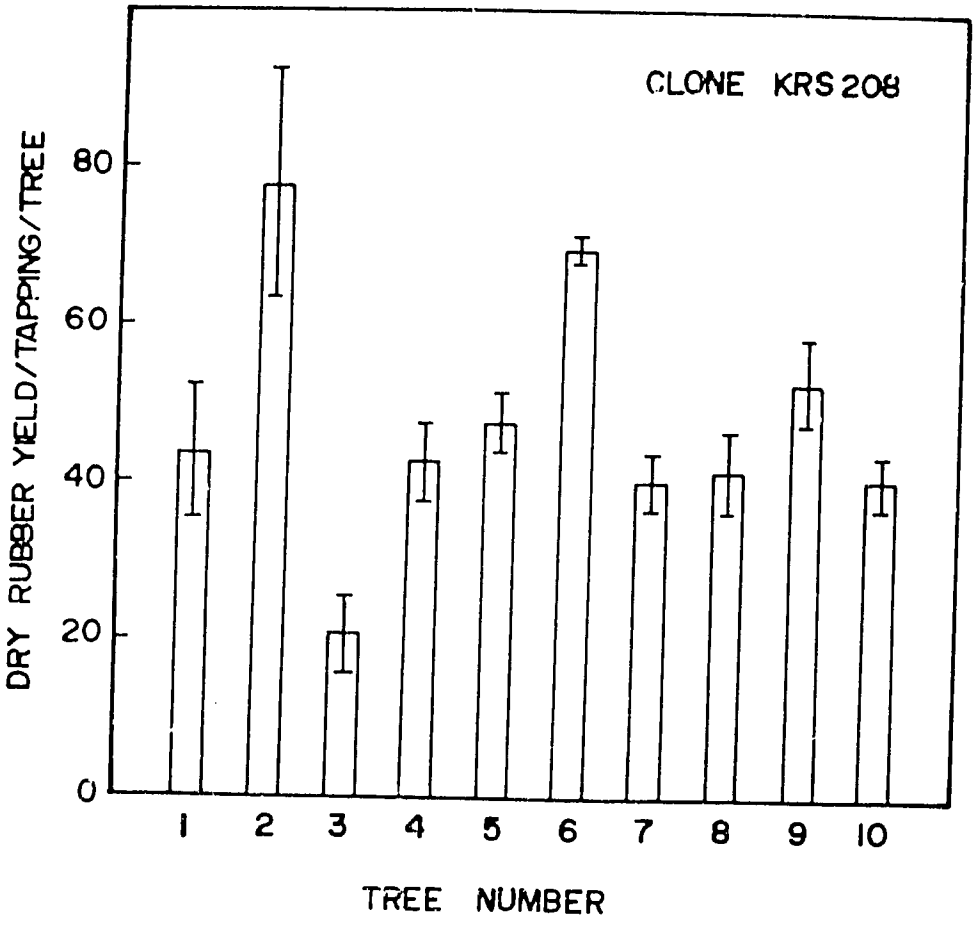

Fig.4. Intraclonal variation of rubber yield per tapping per KRS 208 tree. Each value is the mean ± SD of 5 tapping measurements.

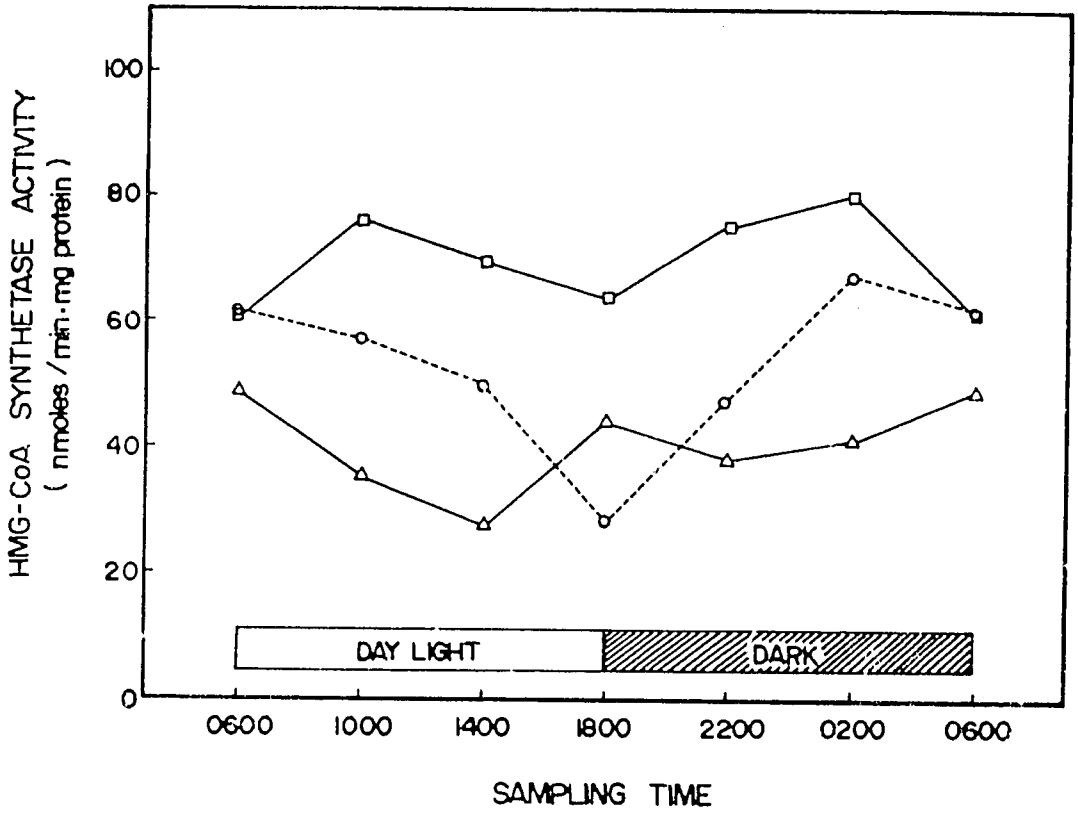

Fig.5. Diurnal variation of HMG-CoA synthetase activity in *Hevea* latex clones RRIM 600 (□—□), KRS 21 (△—△) and GT 1 (○--○). Each point was average of two value obtained from different tappings.

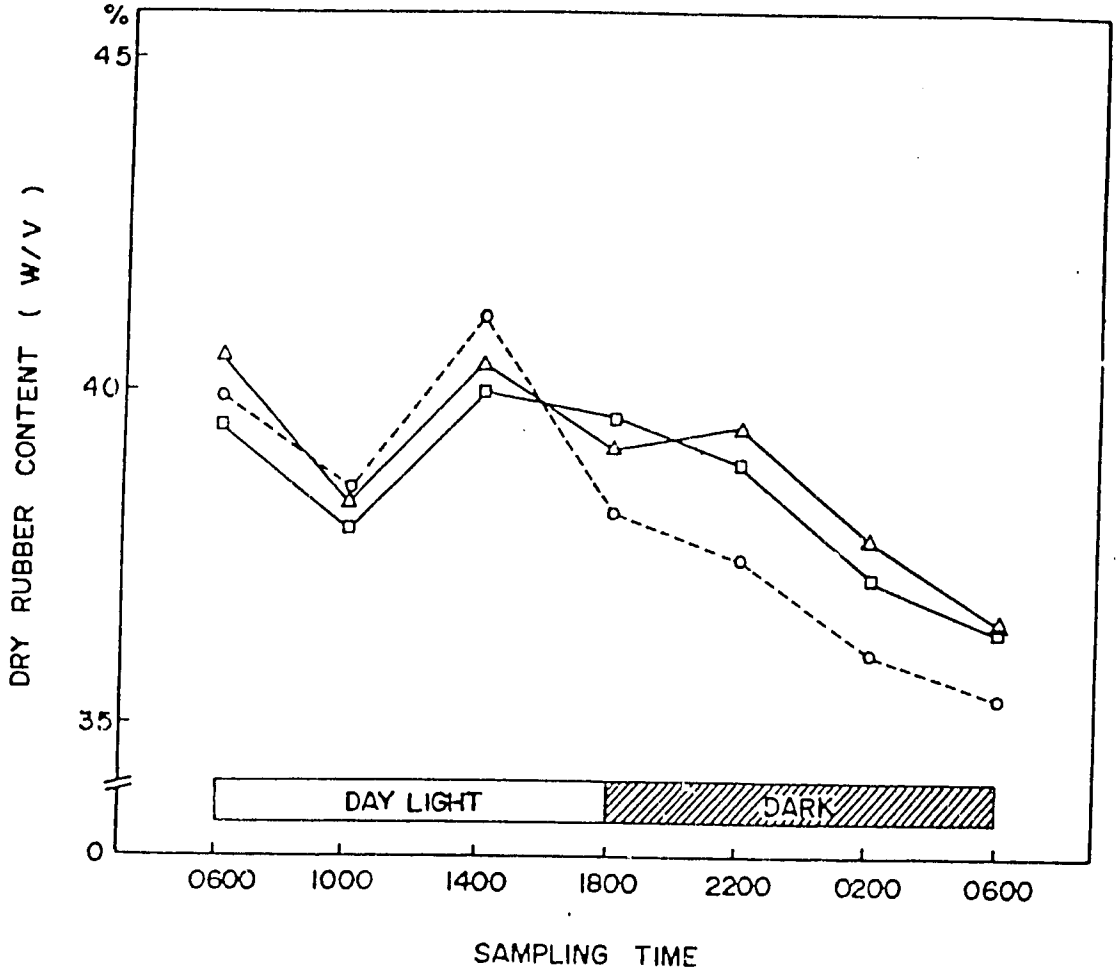

Fig.6. Diurnal variation of percentage of rubber content (w/v) in *Hevea* latex of clones RRIM 600 (□—□), KRS 21 (△—△) and GT 1 (○--○). Each point was the average of two values obtained form different tappings.

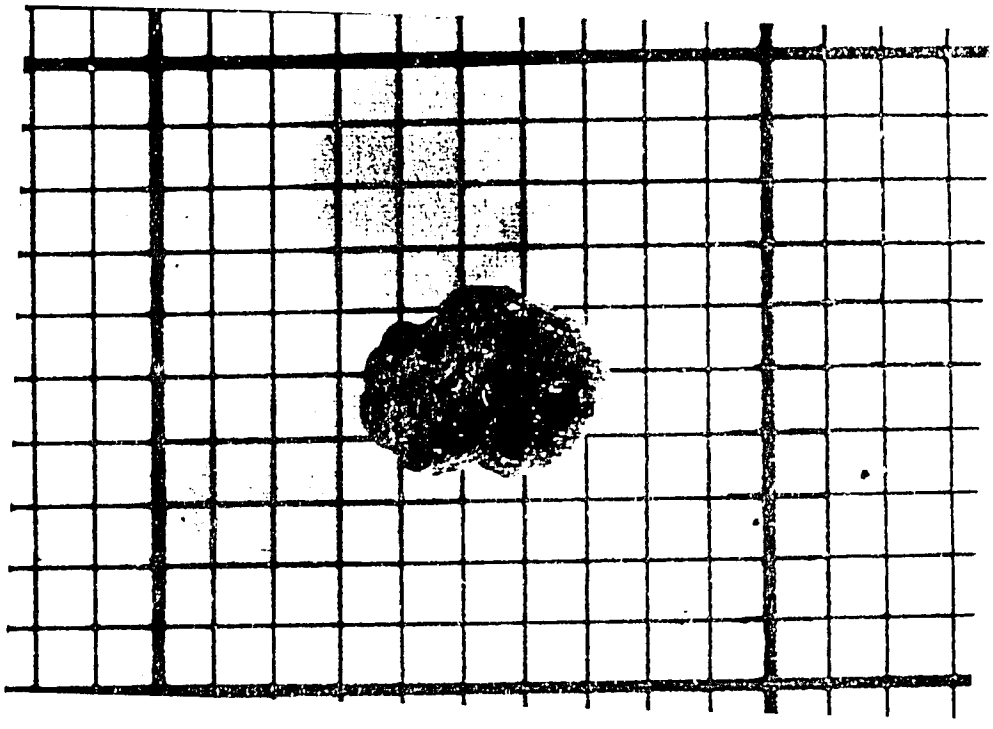

Fig.7. An example of callus which changed colour from yellowish green into brown and died following the 4th weeks as indicated by asterisks in Table 6. There are several phenol-blackened spots on callus.

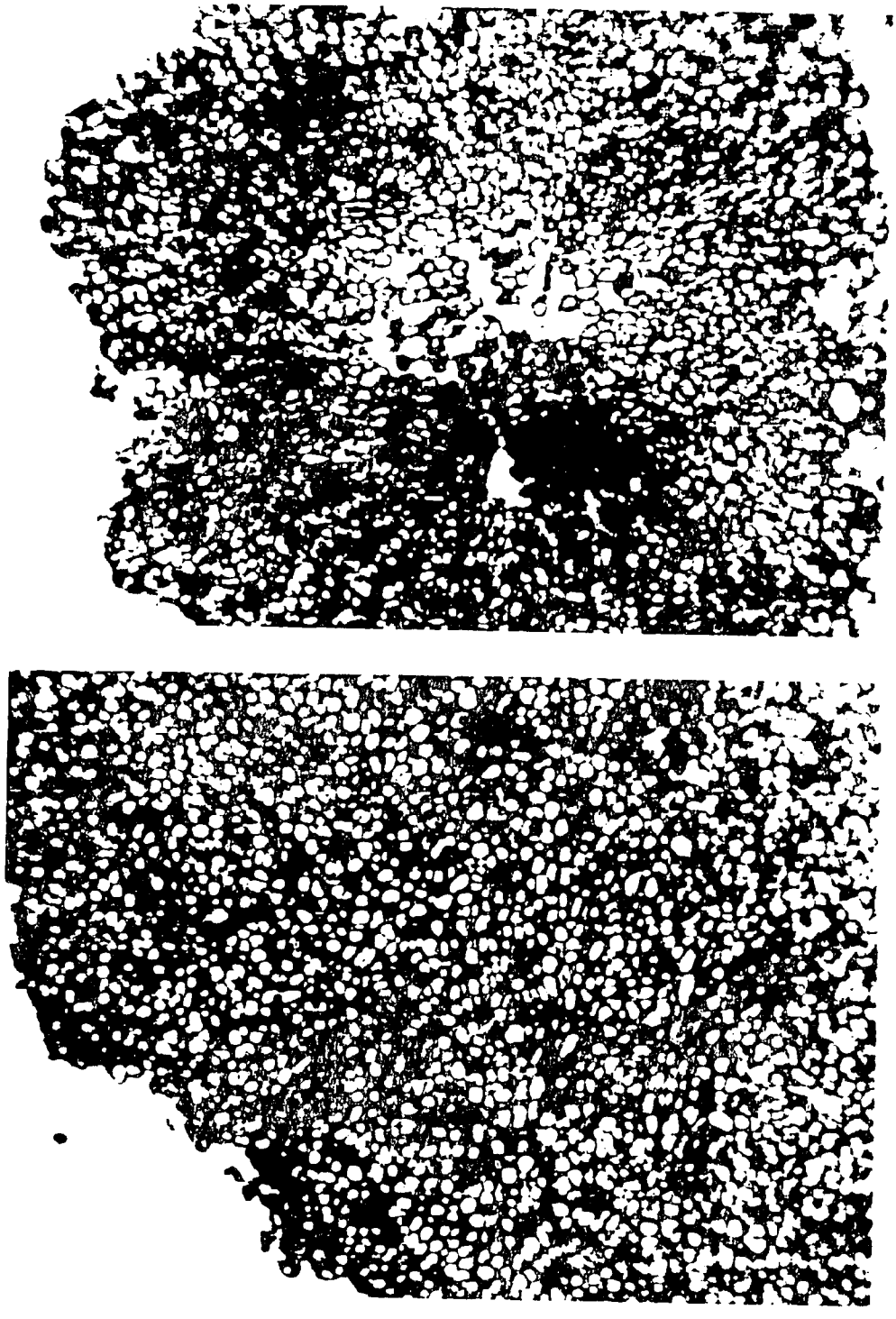

Fig.8. a, b Cross-section of callus from Fig.7. Colour of cells arised from phenol-blackened spots on callus were redish purple while normal cells were greenish blue. Callus appears to be compact but not embryogenic.

Table 1

Correlation studies between HMGR activity and rubber yield from latex of low intermediate, high yield RRIM 600 trees.

Experimental date	RRIM 600 tree number	Dry rubber Yield[1]	Total HMGR activity[2]	Latex HMGR activity[3]	Ranking		
					Dry rubber yield	Total HMGR activity	Latex HMGR activity*
26 Aug'87	8	NA	235 ± 13	5.63 ± 0.26	NA	L	L
	4		340 ± 15	7.71 ± 0.13		M	M
	7		714 ± 4	8.55 ± 0.07		H	H
14 Sept'87	8	18.06	630 ± 29	9.66 ± 0.31	L	L	L
	4	29.70	823 ± 48	9.97 ± 0.30	M	M	M
	7	72.52	2291 ± 1 (r = 0.99)	11.29 ± 0.00 (r = 0.99)	H	H	H
16 Sept'87	8	21.02	690 ± 3	10.99 ± 0.37	M	M	L
	4	19.29	555 ± 16	12.52 ± 0.32	L	L	M
	7	46.93	1507 ± 19 (r = 0.99)	13.41 ± 0.03 (r = 0.86)	H	H	H
20 Oct'87	8	13.13	623 ± 16	11.75 ± 0.07	L	L	L
	4	22.62	903 ± 17	17.95 ± 0.10	M	M	M
	7	55.12	2434 ± 14 (r = 0.99)	22.89 ± 0.37 (r = 0.99)	H	H	H

1 = gm/tapping/tree, 2 = nmoles MVA formed/min/tapping, 3 = nmole MVA formed/min/ml latex, NA = Not available, L = Low, M = Medium H = High, ( ) = correlations calculated between corresponding rubber yield and total HMGR activity or latex HMGR activity

Table 2

Correlation studies between HMGR activity and rubber yield from latex of low, intermediate and high yielding KRS 208 trees.

Experimental date	KRS 208 tree number	Dry rubber Yield[1]	Total HMGR activity[2]	Latex HMGR activity[3]	Ranking		
					Dry rubber yield	Total HMGR activity	Latex HMGR activity*
26 Aug'87	3	NA	153 ± 1	5.97 ± 0.16	NA	L	L
	5		484 ± 11	6.76 ± 0.17		M	M
	6		616 ± 61	6.86 ± 0.01		H	H
14 Sept'87	3	9.15	107 ± 2	4.16 ± 0.28	L	L	L
	5	43.66	1149 ± 20	7.34 ± 0.10	M	M	M
	6	106.24	2274 ± 52 (r = 0.99)	9.21 ± 0.36 (r = 0.98)	H	H	H
20 Oct'87	3	9.89	172 ± 5	9.04 ± 0.05	L	L	L
	5	25.26	661 ± 10	12.89 ± 0.59	M	M	M
	6	67.97	1762 ± 6 (r = 0.99)	15.56 ± 0.16 (r = 0.96)	H	H	H

1 = mg/tapping/tree, 2 = nmole MVA formed/min/tapping

3 = nmole MVA formed/min/ml latex, NA = Not quailable

L = Low, M = Medium H = High, ( ) = correlations calculated between corresponding rubber total HMGR activity or latex HMGR activity

Table 3

Preliminary evaluation for rubber yield on new *Hevea* germplasms

Sample no.	Material source	Clonal code	Yield index*
1	7/02/81	1/22	C^+
2	RO/PB/1	2/35	D^-
3	"-----"	2/36	D
4	"-----"	2/38	D^+
5	"-----"	2/49	C
6	"-----"	2/51	C^+
7	"-----"	2/59	D^+
8	"-----"	2/61	C^+
9	"-----"	2/68	C
10	"-----"	2/69	D^-
11	"-----"	2/83	D
12	RO/PB/2	3/19	D^-
13	"-----"	3/27	C
14	"-----"	3/70	C^-
15	"-----"	3/71	B
16	"-----"	3/78	C^-
17	"-----"	3/87	C
18	"-----"	3/95	D^+
19	"-----"	3/144	C
20	"-----"	3/151	B
21	"-----"	3/165	C
**22	"-----"	3/201	A^+
23	"-----"	3/21	C^+
24	"-----"	3/23	C
25	"-----"	3/26	C
26	"-----"	3/28	B
27	"-----"	3/301	C^+
28.	"-----"	3/351	E
29	"-----"	3/382	D
30	AC/T/3	6/47	B^+

Table 3 (continue)

Sample no.	Material source	Clonal code	Yield index*
31	MT/C/4	7/72	B^-
32	"-----"	7/85	D
33	"-----"	7/114	-
34	MT/C/7	82	E
35	MT/C/2	10/14	D
36	"-----"	10/36	E
**37	"-----"	10/39	A^-
38	"-----"	10/41	E
39	"-----"	10/43	C
40	"-----"	10/48	B
41	"-----"	10/51	D
42	"-----"	10/52	D
43	"-----"	10/54	D
44	"-----"	10/59	D
45	"-----"	10/60	E
46	"-----"	10/61	C
47	"-----"	10/64	D^+
48	"-----"	10/66	B^+
49	"-----"	10/87	D
50	"-----"	10/92	C^+
51	"-----"	10/93	E
52	"-----"	10/94	B
53	"-----"	10/95	C
54	"-----"	10/98	D^+
55	"-----"	10/99	C^+
**56	"-----"	10/108	A
57	"-----"	10/109	B
58	"-----"	10/131	C
59	"-----"	10/144	B
60	"-----"	10/145	D^+
61	"-----"	10/172	D
62	MT/C/6	11/141	A

Table 3 (continue)

Sample no.	Material source	Clonal code	Yield index*
63	MT/C/5	12/30	D
64	"-----"	12/122	D
65	"-----"	12/124	C^+
66	"-----"	12/126	E
67	AC/T/4	13/5	C^+
68	"-----"	13/120	B
69	MT/C/68	14/12	B^-
70	MT/C/3	16/41	B^+
**71	MT/C/1	18/5	A
72	"-----"	18/7	C^+
73	"-----"	18/8	C
74	"-----"	18/79	B
75	"-----"	18/95	C^+
**76	"-----"	18/99	A
77	MT/C/1	18/101	B^+
78	"-----"	18/116	C^-
79	RO/OP/4	20/103	D
80	RO/J/5	33/42	E
81	"-----"	33/45	E
82	"-----"	33/48	C^+
83	"-----"	33/49	D
84	"-----"	33/51	-
85	"-----"	33/52	C
86	"-----"	33/53	D^-
87	"-----"	33/54	D
88	"-----"	33/55	D
89	"-----"	33/56	D
90	"-----"	33/57	E
91	"-----"	33/58	C
92	RO/C/9	23/110	B

Table 3 (continue)

Sample no.	Material source	Clonal code	Yield index*
93	RO/C/9	23/110	B^+
94	"-----"	23/120	C
95	"-----"	23/121	C
96	"-----"	23/129	C^+
97	"-----"	23/142	C^-
98	"-----"	23/150	B^+
99	"-----"	23/152	C
100	"-----"	23/154	B
**101	"-----"	23/158	A
102	"-----"	23/160	C^+
103	"-----"	23/163	C^+
104	"-----"	23/167	-
105	"-----"	23/174	B
**106	"-----"	23/177	A
107	"-----"	23/180	B^-
108	"-----"	23/182	C^+
109	"-----"	23/183	E
110	"-----"	23/184	C
111	"-----"	23/194	C^-
112	"-----"	23/199	B
113	"-----"	23/202	C
114	"-----"	23/206	B^+
153	MT/VB/25A	57/53	D
155	"-----"	57/58	E
157	"-----"	57/71	D
159	"-----"	57/19	C^-
115	RO/C/9	23/212	B^-
116	"-----"	23/216	D
117	"-----"	23/219	C^+
118	"-----"	23/225	D
119	"-----"	23/234	C
120	"-----"	23/236	C

Table 3 (continue)

Sample no.	material source	Clonal code	Yield index*
121	RO/C/9	23/239	D
122	"-----"	23/242	D
123	"-----"	23/267	E
124	"-----"	23/277	E
**125	"-----"	23/294	A^-
**126	"-----"	23/294	A^-
127	"-----"	23/301	C
128	"-----"	23/318	-
129	"-----"	23/329	C^+
130	"-----"	23/346	-
131	"-----"	23/353	-
132	"-----"	23/382	C
**133	"-----"	23/386	A
134	"-----"	23/393	B^+
135	"-----"	23/396	$C-C^+$
136	"-----"	23/398	C
137	"-----"	23/405	A^-
138	"-----"	23/411	$C-C^+$
139	"-----"	23/413	C^-
140	"-----"	23/416	-
141	RO/C.9	23/423	C
142	MT/VB/25A	57/2	C
143	"-----"	57/4	C^-
144	"-----"	57/5	E
145	"-----"	57/8	D-E
146	"-----"	57/14	D
148	"-----"	57/32	C
149	"-----"	5Y/37	C^-
150	"-----"	57/44	C^+

Table 3 (continue)

Sample no.	material source	Clonal code	Yield index*
151	MT/VB/25A	57/45	C-D
152	"-----"	57/50	E
154	"-----"	57/57	D
156	"-----"	57/69	D^+
158	"-----"	57/76	C^-
160	"-----"	57/81	C^-

*Yield index was graded by adjusting from amout of latex being excreted from rubber leaflet upon cutting through leaf blade parallel to midrib vein. Grades A to E were given for high to low latex yield obtained.

**Selected for futher yield screening by HMGR assay.

Table 4

Studies on latex HMGR activity from new *Hevea* germplasms

Material sources	Clonal codes	HMGR activity per ml latex
Wickham	RRIM 600	5.17
RO/PB/1	3/201	NA
MT/C/2	10/39	1.46
MT/C/2	10/108	2.78
MT/C/1	18/5	6.73
MT/C/1	18/99	2.36
RO/C/9	23/158	4.42
RO/C/9	23/177	1.59
RO/C/9	23/294	1.50
RO/C/9	23/294	NA
RO/C/9	23/386	1.68

NA = not avialable due to insufficient amount of latex obtained by microtapping.

Table 5

Correlation studies on latex MVA kinase and HMG-CoA synthetase activity levels and rubber yield in high, medium and low yielding rubber trees.

Source of latex	MVA kinase activity*	HMG-CoA Synthetase activity*	Ranking	
			MVA kinase	HMG-CoA syntase
^{H}RRIM 600	60.45	59.50	L	L
^{M}RRIM 600	78.59	62.10	H	M
^{L}RRIM 600	75.97	72.20	M	H
^{H}KRS 208	60.51	47.10	L	L
^{M}KRS 208	63.14	51.60	M	M
^{L}KRS 208	76.11	53.70	H	H

*Activity in C-serum of latex collected between 15th-20th min. after tapping, expressed as nmol/min/mg. protein.
H,M and L represent latex obtained from high, medium and low yielding trees accordingly.

Table 6

Callus culture of male flowers from various *Hevea* clonal types.

Inoculation date	Clones	Culture media	Number of samples	Callus growth	
				wks	mm
9/10/87	RRIM 600	MB	20	3	2.5
				*4	2.7
9/18/87	RRIM 600	MB	2	2	0.51
				4	1.03
				6	1.50
				8	2.06
				10	2.57
				*12	3.0
8/20/87	PB 310	MB	40	1	1.0
				2	1.5
				*4	1.85
				9	2.5
11/4/87	PB 310	MB	24	2	0.58
				*4	1.12
11/13/87	PB 310	MZZ(MZ)	30	2	0.71
				*4	.9
10/15/87	RRIC 6	MB	12	2	0.69
				4	1.47
				6	2.25
10/8/87	GT 1	MB	16	2	0.45
				4	0.81
				*6	1.20

* Callus began to change from yellowish green into brownish colour and died in the following weeks.

Table 7

Callus growth under different types of media.

Inoculation date	Clones	Culture media	Number of samples	Callus growth		1st transfering date	Culture media	Callus growth	
				wks	mm.			wks	mm.
11/17/87	RRIC 6	MZZ (ZM)	10	2	0.57	12/3/87	MLLH	2	0.9
11/19/87	RRIC 6	MZZ (ZM)	25	2	0.58	12/3/87	MLLH	2	0.9
11/20/87	RRIC 6	MZZ (ZM)	38	2	0.70	12/4/87	MLLH	2	0.8
11/24/87	RRIC 6	MZZ (ZM)	24	2	0.74	12/8/87	MLLH	2	1.0
11/25/87	RRIC 6	MZZ (ZM)	20	1	0.56	12/9/87	MLLH	2	0.9
11/26/87	RRIC 6	MZZ (ZM)	32	1	0.58	12/10/87	MLLH	2	1.0
11/27/87	RRIC 6	MZZ (ZM)	40	2	0.8	12/11/87	MLLH	2	1.0
12/1/87	RRIC 6	MZZ (ZM)	47	2	0.9	12/14/87	MLLH	2	1.0