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CLONAL VARIATIONS IN THE ACTIVITY OF 3-HYDROXY-3-METHYL GLUTARYL-CoA REDUCTASE IN BARK OF *HEVEA BRASILIENSIS*

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HMG-CoA reductase activity in the bark of *Hevea brasiliensis* was estimated in four clones with contrasting yield characteristics. Significant difference in bark enzyme activity was observed between the high yielding and low yielding clones.

Key words – *Hevea brasiliensis*, Clonal variation, HMG-CoA reductase, Rubber biosynthetic capacity.

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INTRODUCTION

Two major factors limiting latex production in *Hevea brasiliensis* are flow characteristics which govern the quantity of latex obtained on tapping and the *in situ* regeneration of latex between two successive tappings. Although the pathway of rubber biosynthesis, as far as the intermediates and enzymes are concerned, is well understood but not much work has been done on the factors controlling regeneration of latex and thereby that of rubber. A knowledge of the regulatory mechanisms might find immediate practical application in controlling or stimulating rubber production in the *Hevea* tree at least to certain extent. A look into the activities of the enzymes involved in rubber biosynthesis from acetate shows that HMG-CoA reductase (EC 1.1.1.34) activity, which is responsible for the formation of mevalonic acid, is much lower and that this enzyme may be a limiting factor in rubber biosynthesis (Lynen, 1969). Reports on the biosynthetic rates of rubber regeneration in the bark during the interval between successive tappings are scanty. A study was therefore undertaken to estimate the HMG-CoA reductase activity in the bark in the drainage area in four clones, two each belonging to high yielding and low yielding groups, in an attempt to find out any possible relationship between the activity of this enzyme and yield of rubber. A quick indirect method of estimating HMG-CoA reductase activity was employed with an objective of ascertaining the suitability of such a method for large scale screening.

MATERIALS AND METHODS

Bark samples were collected in the dry season (March-April) of 1989 from six trees each of clones RRII 105, PB 235 (high yield-

ing), Ch 4 and Pil B 84 (low yielding) from a completely randomised planting in the germplasm garden. The trees were in the second year of tapping in BO 1 panel. Bark samples were taken from the drainage area in the vicinity of the ends of the tapping cut. The samples were collected in polythene bags in ice on the day of tapping, 30 to 60 min after complete cessation of latex flow. Activity of hydroxy methyl glutaryl coenzyme A reductase (HMG-CoA reductase EC 1.1.1.34) was assayed by determining the ratio of HMG-CoA to mevalonic acid (Rao and Ramakrishnan, 1975). Soft tissues of the bark were homogenised in one per cent saline arsenate and diluted with an equal volume of perchloric acid (50 ml litre^{-1}). The extract was centrifuged at 3000 rpm for 15 min. One ml of the supernatant centrifugate was treated with 0.5 ml of 2 M freshly prepared hydroxylamine in water. In the case of HMG-CoA, hydroxylamine reagent mixed with an equal volume of 4.5 M sodium hydroxide solution was used whereas, in the case of mevalonate, hydroxylamine reagent mixed with equal volume of water was used. The solutions were mixed and kept for 5 min and 1.5 ml of ferric chloride was added. After 10 min the colour was read at 540 nm against a similarly treated blank. The ratio of HMG-CoA to mevalonate was taken as an index of enzyme activity, a low ratio indicating high enzyme activity and *vice versa*. Correlations were worked out between bark enzyme activity, rubber yield and drc.

RESULTS AND DISCUSSION

The results (Table 1) indicate that there is significant clonal variation in the activity of HMG-CoA reductase in bark of high and low yielding clones. Maximum activity was observed in high yielding clones PB 235 and RRII 105. HMG-CoA mevalonate ratios were found to be negatively correlated with dry rubber yield ($r = -0.76^{**}$) and dry rubber content ($r = -0.47^{*}$). 3-hydroxy-3-methyl glutaryl-CoA reductase plays a key role in the regulation of cholesterol biosynthesis in animals, but its role in the regulation of isoprenoid biosynthesis in plants is not well understood (Lalitha and Ramasarma, 1985).

Table 1. **Clonal variations in HMG–CoA reductase activity, dry rubber content and dry rubber yield (mean values)**

Clone	* HMG–CoA reductase activity (HMG–CoA/Mevalonate)	drc (%)	Dry rubber yield (g tree^{-1} tap^{-1})
Ch 4	2.26	40.6	25.90
Pil B 84	2.70	37.2	6.80
RRII 105	1.55	44.9	67.90
PB 235	1.52	43.9	74.42
CD 0.05	0.174	1.26	7.45

* Higher ratio of HMG-CoA/Mevalonate indicates lower enzyme activity.

Earlier studies (Hepper and Audley, 1969 and Sipat, 1982) have attributed HMG-CoA reductase activity to the microsomal fraction derived from the endoplasmic reticulum of laticifers. Since the enzyme is associated with endoplasmic reticulum of latex vessel it is unlikely to be completely represented in the free polysomes found in exuded latex (Kekwick, 1986). In the light of the above findings it would appear that measurement of enzyme activity in latex may not give a true representation of the *in vivo* level of the enzyme. However, a correlation between enzyme activity in latex and dry rubber yield has been reported by Wititsuwannakul and Sukonrat (1984).

In the present study, since soft tissues of the bark, which contain the truly productive laticifers was used, the enzyme activity obtained might indicate the *in vivo* situation. The positive correlation obtained between the bark enzyme activity and dry rubber yield suggests the possibility of utilizing this method also for characterisation of clones for high yield potential. While the indirect method employed in the present study does not give the actual quantity of the enzyme present in the tissue, it gives reasonable indication of the rate of enzyme activity which would be useful when the number of samples to be screened is high. Further confirmatory investigations are necessary using radiotracer methodology to quantify the enzyme activity.

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