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IDENTIFICATION OF TWO POLYPEPTIDES IN THE LUTOID MEMBRANE OF HIGH YIELDING CLONES OF *HEVEA BRASILIENSIS*

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ABSTRACT

Intact lutoids were isloted from latex samples of high and low yielding clones of *Hevea brasiliensis*. Membranes were prepared by osmotic lysis and extracted proteins were assayed and subjected to SDS - Polyacrylamide gel electrophoresis. Higher protein content was observed in the membrane from high yielding clones compared to low yielders and two polypeptides with molecular weight 63.1 and 79.4 KD were identified specifically for the high yielding clones. The role of these polypeptides in the stability of the membrane and its possible application in early evaluation of high yielding clones are discussed.

INTRODUCTION

Latex lutoids are single membrane bound micro vacuoles with lysosomal characteristics (Pujarniscle, 1968), which accumulate and compartmentalise numerous ions, cationic proteins, hydrolases and retains ATP ase and acid phosphatase activity. Breakage of the membrane and release of the bound materials cause partial coagulation of rubber particles and microflocs are formed. Cessation of latex flow in tapped trees are caused by formation of latex coagulum in the cut ends of the latex vessels. The coagulum acts as physical barriers to latex flow and the phenomenon is called latex vessel plugging. The nature of the membrane surrounding lutoids is therefore very important. Dupont *et. al.* (1975) studied the phospholipid and fatty acid composition of the membrane and characterised by a high content of phosphatidic acid. Much less information is available with respect to protein content, function and organisation in the membrane. In the present study, lutoid membranes were prepared from latex samples of high and low yielding clones and proteins were characterised using SDS-PAGE to find out whether these changes could be used as an early prediction parameter for high yield.

MATERIALS AND METHODS

Latex samples were collected from four trees each of clones RRII 105, PB 135, PB 215, PB 217 , (high yielding), Ch4, Pil B 84, Tjir 16 and ch 29 (low yielding) from the germplasm garden.

Membrane Isolation

Fresh latex was centrifuged at 23,000 rpm for 45 min at 4°C. Supernatent serum and rubber fractions were discarded and the pellet suspended in 5 volumes of 50mM Hepes - Tris buffer (pH 7.0), 300 mM mannitol. The crude lutoid fraction was washed three times with the same buffer. Lutoids so obtained were subjected to osmotic shock at 4°C in a medium containing 50 mM Hepes, Tris (pH 7.0). Membranes were separated by centrifugation at 30,000 g for 30 min at 4°C and washed two times in the same medium (Xavier Gidrol *et al.* 1988).

Analysis of Membrane Proteins

Membranes were solubilised in a buffer containing 62.5 mM Tris- HCl pH 6.8, 12% (w/v) glycerol, 2% (w/v) SDS and 5% (v/v) 2-mercaptoethanol and the dispersed samples were heated at 100°C for 3 min. (Howard Evans,

1979). Protein contents of the samples were assayed by the method of Lowry *et. al.* (1951). using BSA as standard.

Gel electrophoresis

SDS-PAGE was performed on slab gels (175 x 162 x 1mm) using the SDS buffer system described by Laemmli (1970). Separating gel contained 10% (w/v) polyacrylamide (30:0.8 acrylamide and N,N' methylene bis acrylamide), 1% (w/v) SDS and 0.375 M Tris (pH 8.8). The stacking gel was 3% acrylamide, 0.14% bis and 0.138 M Tris (pH 6.8). Electrophoresis was performed at 20 mA at 18°C for 5 hrs with an upper and lower tank buffer of 0.25 M Tris and 0.19 M glycine (pH 8.3). Aliquots equivalent to 50 mg proteins were loaded in each lane. The bands were visualised by 0.1% (w/v) coomassie brilliant blue R-250. Molecular weights were estimated from the position of prestained marker proteins which include Bovine Albumin (66 KD), Ovalbumin (45 KD), Pepsin (34 K D) Trypsinogen (34 KD) and lysozyme (14 KD).

RESULTS AND DISCUSSION

The results (Table I) indicate that the protein content of the lutoid membrane of high yielding clones were significantly high when compared to low yielders. Tata (1980) studied the distribution of proteins between ultracentrifuged fractions of latex and showed that 44% of the proteins of bottom fraction was membrane proteins from lutoids. A higher concentration of proteins in the lutoid membrane may possibly contribute to the stability of lutoids which is one of the major factors determining rubber yield. It has also been shown that the greater stability of lutoids in high yielding clones may be associated with high content of phospholipids and triglycerides in the lutoid membrane (Usha Nair *et. al.*, 1993).

SDS-PAGE profile and the major changes in protein composition in high and low yielding clones were shown in Table II. Electrophoresis resolved twelve polypeptide bands with mol. wt. ranging from 13-166 KD. A general decrease in the content of proteins 166 and 151.4 KD and absence of 79.4 and 63.1 KD proteins were observed in the low yielding clones (Fig. 1 a,b). Further more, a 58.8 KD polypeptide in high yielding clones could not be discerned in the low yielding group. The specific polypeptides identified in high yielding clones are indicated by arrows in the figure. No difference in the banding pattern was observed when the low yielding trees are subjected to ethrel (2- chloro ethyl phosphoric acid, commonly used to stimulate the production of latex) treatment. The polypeptide profiles were reproducible in all the gels associated with each clone.

Table I. Protein content (mg g^{-1} dw) of lutoid membrane in high and low yielding clones of Hevea

Clone	Protein content
RRI 105	16.69
PB 235	15.56
PB 217	17.58
PB 215	13.49
Ch 29	10.88
Tjir 16	10.74
Ch 4	9.68
Pil B84	9.34
CD	1.79

So far, two antagonistic enzyme systems AT Pase (EC 3.6.1.3) and NADH cytochrome c-reductase (E.C. 1.6.99.3) located on the lutoid membrane have been studied in latex. Marin and Komor (1984) studied the subunit composition of purified ATP ase extracted with dichloromethane from the lutoid tonoplast and showed polypeptides with mol. wts. of 110, 68, 24 and 12 KD.

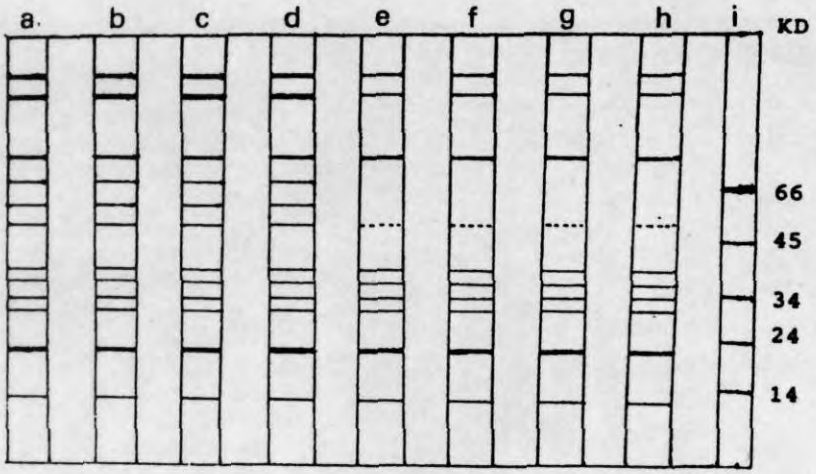

Fig. 1a. **SDS-PAGE profile of proteins extracted from lutoid membrane of high and low yielding clones**

Lanes : a, b, c, d - RRII 105, PB 235, PB 215, PB 217
e, f, g, h - Ch 4, Pil B 84, ch 29, Tjir 16
i - Molecular weight markers.

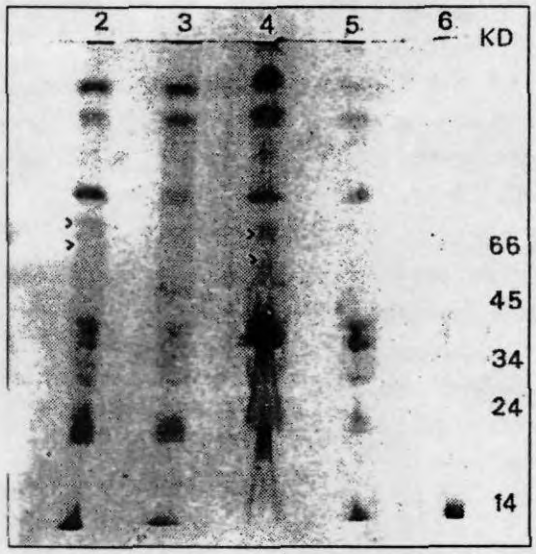

Fig. 1 b. **SDS-Polyacrylamide Coomassie blue stained gel of proteins extracted from lutoid membrane of high and low yielding clones.**

Lane : 1 & 6 : Mol. wt. markers
2, 3, 4 : High yielding clones
5 : Low yielding clone
50 µg proteins were applied per lane. Arrows indicate additional polypeptides.

Table II. Lutoid membrane polypeptides resolved by SDS -PAGE and changes in their composition in high and low yielding clones.

Peptide fraction	Mr (KD)	High yielding clones	Low yielding clones
1	166.00	+	–
2	151.40	+	–
3	89.00	+	+
4	79.43	–	0
5	63.10	–	0
6	58.80	–	–
7	39.80	+	+
8	37.15	+	+
9	33.88	–	–
10	28.18	–	–
11	22.30	+	+
12	13.80	+	+

+ increase in content, – decrease 0 - absence of bands.

The additional bands 79.4 and 63.1 KD may specifically be synthesised or repressed with some clones. The changes in the profile may be considered as a criteria for identifying high yielders. If this pattern is expressed in the latex of young trees, it would be possible to utilize this parameter for early prediction of productivity. Immunochemical characterisation of these proteins are needed to study the specific role of these proteins in the mechanism of latex vessel plugging and therefore yield.

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