

## A STUDY ON CHARACTERIZATION OF HEVEA CLONES BY SERUM PROTEIN PATTERNS

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### SUMMARY

Protein patterns in the A-serum (acid serum) of *Hevea brasiliensis* were investigated. Altogether 72 clones, commercially cultivated in Sri Lanka were screened for their protein patterns by PAGE electrophoresis using Tris-glycine buffer at pH 8.5, and 7.5% small pore gels. A wide range of protein bands varying from a minimum of 14 bands in RRIC 51 and RRIC 129 to a maximum of 26 bands in WR 101 was observed. A typical diagram comprising of 33 bands of all the clones investigated and a summary of the distribution of proteins in all the clones were prepared. The 72 clones investigated were divided into 8 main groups based on their serum protein patterns.

### INTRODUCTION

New clones of *Hevea brasiliensis*, with improved characteristics such as resistance to diseases and high yield potential are constantly being increased in NR producing countries. The identification and characterization of this wide range of old as well as new *Hevea* clones, with various characteristics for various purposes, are of great importance and have received considerable attention from *Hevea* researchers in recent years. The existing methods of identification of clones are based on morphological and physiological attributes which are not entirely satisfactory.

Several chemical and biochemical markers are successfully being used in identification of different cultivars of a number of other crops. Amongst these markers are protein patterns in wheat (Bushuk and Zillmann, 1978, Zillmann and Bushuk-1979), leaf flavonoids in soybeans (Buttery and Buzzel, 1973) flavonoids in flowers of New Guinea white (Asen, 1977), protein analysis in peach (Carter and Brock, 1980), pollen proteins in *Zea mays* (Greenhouse et al 1982) in olive (Pontikis et al 1980), and phenols in poinsettia (Werner and Sink, 1977).

The clonal variation in discolouration of *Hevea* latex by calcium chloride test observed by Babilloff (1931), is not specific enough for distinguishing clones. The clonal characterization based on the characters of young budded trees, which are constant for each clone such as shape of storeys, length and shape of the leaf stalks arrangement of leaves etc, suggested by Mann and Sharp (1933), again relies on morphological criteria. Silva and Satchuthananthavale (1962,1963) have also made an attempt to give a description of some locally improved clones by examining their vegetative characters. The possibility of using protein patterns for clonal identification was first investigated by Walujono and Suseno (1973) using polyacrylamide gel electrophoresis. Yeet et al (1977) have also investigated the isoenzymes in latex serum of some *Hevea* cultivars and reported in favour of using them in clonal identification.

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The present investigation was carried out in the same lines attempted by Walujono and Suseno (1973), with some modifications in the electrophoretic procedure and with a view to screening all the *Hevea* clones planted on a commercial scale in Sri Lanka.

#### EXPERIMENTAL

Of about 100 clones planted commercially in rubber estates of Sri Lanka, 72 clones were selected for the study of their protein patterns in A-serum depending on their availability and extent of cultivation. Latex samples were collected from 5 different trees of each clone which were in alternate daily half spiral tapping (S/2 d/2 system).

Preparation of A-serum (Acid serum) was based on the method described by Walujono and Suseno (1973). About 25 ml of latex was collected directly from the tree into 50 ml glass beakers in ice. To 20 ml of latex, 10ml of 2% v/v acetic acid was added. The soft coagulam formed after about 2h was pressed by a glass rod and clear serum was collected in glass vials. These samples were kept in the refrigerator until further handling. Proteins in A-serum were separated by polyacrylamide gel electrophoresis (PAGE).

Electrophoresis was carried out with acrylamide 7.5% small pore, and Tris-glycine buffer, pH 8.5. A constant current of 5mA/tube for about 50 min was applied. Bromophenol blue was used as the front marker. The gels were stained as described by Chrambach *et al* (1967) with Coomassie Brilliant Blue R 250 in 12.5% trichloroacetic acid. The gels were removed from the staining solution after 48 h and preserved in 7% acetic acid. The protein patterns were recorded and photographed by placing on an illuminating box.

#### RESULTS AND DISCUSSION

The results of this study show that latex serum (A-serum) can be successfully used for obtaining a protein pattern fairly characteristic for each clone. In addition to the work of Walujono and Suseno (1973), the previous reports also show that *Hevea* latex contains a reasonably higher protein content that is sufficient for an investigation of this nature (Blackley 1966; Archer *et al* 1969). Yeet *et al* (1977) showed the presence of number of isoenzymes in latex serum and the protein pattern differences among some *Hevea* species and clones. Jacob *et al* (1978) too, obtained 20 different enzymes and 7 different enzymes in C-serum (centrifuged serum) and B-serum (bottom fraction serum) of *Hevea* respectively using PAGE electrophoresis. They used 5 clones and compared zymograms of C-serum of 4 enzymes namely alcohol dehydrogenase, esterase, phosphoglucomutase and peroxidase for clone identification using the number and intensity of the bands.

A diagrammatic representation of a typical gel for all the 72 clones investigated was prepared and is given in Fig. 1, which contains a total of 33 protein bands. The distribution of protein bands among various clones was examined by comparing all the clones individually with the typical protein pattern and a summary of this distribution pattern of protein bands is given in Table 1 which could be used as a guide in identification of *Hevea* clones. Fig. 2 shows the electropherograms of some of the popular clones in Sri Lanka.

A summary of distribution of different protein bands in clones of respective categories is given in Table 2. A summary of important differences between protein

Table 1. Distribution of protein bands in A - serum of different clones, obtained by polyacrylamide gel electrophoresis

Clone	Protein band number																																	Total	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33		
PB 86	+	+	+	+	-	+	-	+	+	+	-	+	+	+	+	-	+	+	-	+	+	-	+	+	-	+	-	+	+	+	+	-	+	24	
RRIC 7	+	-	-	+	-	+	-	+	+	+	+	-	-	+	+	-	-	+	+	-	-	+	-	+	+	-	+	-	+	+	-	-	+	17	
RRIC 22	+	-	-	+	+	+	-	+	+	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	-	+	+	+	-	-	-	+	18	
RRIC 28	+	-	-	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	-	+	-	+	+	+	-	+	-	+	-	-	+	22	
RRIC 36	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	-	-	+	-	-	+	-	+	-	-	+	-	+	+	+	21	
RRIC 37	+	-	-	+	-	+	-	+	+	+	-	-	+	-	+	+	-	+	+	-	+	-	-	+	-	+	-	-	+	-	+	+	+	-	18
RRIC 39	+	-	+	-	-	+	-	+	+	+	-	-	-	-	+	-	-	+	+	-	+	+	-	+	-	+	-	-	+	-	-	-	+	15	
RRIC 41	+	-	+	-	-	+	-	+	+	+	-	+	+	-	+	+	-	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	+	-	18
RRIC 45	+	-	+	-	-	+	-	+	+	+	-	-	-	+	+	+	-	+	+	-	-	+	-	+	+	-	-	+	+	-	+	-	+	18	
RRIC 51	+	-	+	+	-	+	-	+	+	+	-	-	-	-	+	-	-	+	+	-	+	-	-	+	-	+	-	-	+	-	-	-	-	14	
RRIC 52	+	-	+	+	-	+	-	-	+	+	-	-	-	-	+	+	-	+	-	-	+	+	-	+	-	+	-	-	+	+	-	+	+	17	
RRIC 54	+	-	+	+	-	+	-	+	+	+	-	-	+	-	+	+	-	+	+	-	-	+	-	+	+	+	-	-	+	-	-	-	+	17	
RRIC 55	+	-	+	-	+	+	-	+	+	+	-	-	+	-	+	+	+	+	-	-	+	+	-	+	-	+	-	-	+	-	-	-	+	17	
RRIC 60	+	-	+	-	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	-	-	+	-	+	+	+	+	-	+	-	+	22	
RRIC 75	+	-	+	+	+	+	-	+	+	+	-	+	+	-	+	-	-	+	+	-	+	+	-	+	-	+	+	+	+	-	+	-	+	21	
RRIC 76	+	-	-	+	+	+	+	+	+	+	-	+	+	-	+	-	-	+	-	+	-	-	-	+	-	+	-	-	+	-	-	-	-	15	
RRIC 88	+	-	+	-	+	+	+	+	+	+	-	-	+	-	+	+	-	+	-	-	+	-	-	+	-	+	-	-	+	-	+	+	-	18	
RRIC 89	+	+	-	+	+	+	-	+	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	-	+	-	-	+	-	-	-	-	19	

Table 1 Contd.

Clone	Protein band number																																	Total		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33			
RRIC 90	+	-	-	+	-	+	+	+	+	+	+	+	-	+	+	-	-	+	+	+	+	-	-	+	+	+	+	-	-	+	+	+	-	+	22	
RRIC 100	+	-	+	-	-	+	-	-	+	+	-	-	-	+	+	+	-	+	-	+	+	-	-	+	-	+	+	-	-	+	+	+	+	+	18	
RRIC 101	+	-	+	+	-	+	-	+	+	+	-	-	-	+	+	+	+	+	-	+	-	+	-	+	-	+	+	-	-	+	+	-	+	+	19	
RRIC 102	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	-	+	-	+	-	-	+	+	-	+	18	
RRIC 103	+	-	+	+	-	+	-	+	+	+	-	-	-	-	+	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+	+	-	+	+	18	
RRIC 104	+	-	+	+	-	+	-	+	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	22
RRIC 106	+	-	+	+	-	+	-	+	+	+	-	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	22
RRIC 107	+	+	+	+	+	+	-	+	+	+	-	+	-	-	+	+	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	23
RRIC 108	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	22
RRIC 109	+	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	19
RRIC 110	+	-	+	-	+	+	-	+	+	+	-	-	-	-	+	-	+	+	-	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	18
RRIC 111	+	-	+	-	+	+	-	+	+	+	+	-	+	-	+	-	+	+	+	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	19
RRIC 112	+	+	+	-	+	+	-	+	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	21
RRIC 113	+	-	-	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	23
RRIC 114	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	23
RRIC 117	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	+	-	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	20
RRIC 118	+	-	+	+	-	+	+	+	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	21
RRIC 121	+	-	+	-	+	+	+	+	+	+	-	-	+	-	+	-	+	+	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	21
RRIC 122	+	-	+	-	-	+	+	+	+	+	-	-	+	-	+	+	+	-	+	+	-	+	+	-	+	-	+	+	+	+	+	+	+	+	+	20
RRIC 123	+	-	+	-	+	+	-	+	+	+	-	+	-	-	+	+	+	-	+	+	+	+	-	+	-	+	-	+	-	+	+	+	+	+	+	17

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Table I contd.

Clone	Protein band number																																	Total		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33			
RRIC 124	+	-	+	-	-	+	-	+	+	+	-	-	+	-	+	+	-	+	+	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	15	
RRIC 125	+	-	+	-	+	+	-	+	+	+	-	-	+	-	+	+	-	+	+	+	+	+	-	+	-	+	-	-	+	+	+	-	-	+	21	
RRIC 126	+	-	+	-	-	+	-	+	+	+	-	+	+	-	+	-	-	+	+	-	+	-	+	+	+	-	+	-	+	-	-	-	-	-	15	
RRIC 127	+	-	+	-	+	+	-	+	+	+	-	-	+	-	+	+	-	+	+	+	+	+	-	+	+	+	-	-	+	-	-	-	-	+	20	
RRIC 128	+	-	-	-	-	+	-	+	+	+	-	+	-	+	+	+	-	+	-	+	+	-	-	+	-	+	+	+	-	-	+	-	-	+	18	
RRIC 129	+	-	+	-	-	+	-	+	+	+	-	-	+	-	+	+	-	+	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	14	
RRIC 130	+	-	-	+	-	+	-	+	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	+	+	-	-	-	+	-	+	+	+	-	19	
RRIC 131	+	-	-	+	-	+	+	+	+	+	-	-	+	-	+	-	+	+	+	+	-	+	-	+	-	+	+	+	+	+	+	+	+	+	21	
RRIM 513	+	-	+	-	+	+	+	+	+	+	-	-	+	-	+	+	-	+	-	+	+	+	-	-	+	-	+	-	-	+	+	-	+	-	20	
RRIM 600	+	-	-	+	+	+	-	+	+	+	-	-	+	-	+	+	-	+	+	-	-	+	-	+	+	-	+	+	-	+	+	-	-	+	19	
RRIM 603	+	-	-	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	-	+	-	+	-	-	+	+	+	+	+	+	+	+	19	
RRIM 605	+	-	+	+	-	+	-	+	+	+	-	-	+	-	+	-	+	+	-	+	-	-	-	-	+	-	+	-	-	+	-	-	-	+	16	
RRIM 607	+	-	-	+	-	+	-	+	+	+	-	-	+	-	+	+	-	+	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	+	15	
RRIM 612	+	-	-	+	-	+	-	+	+	+	-	-	+	-	+	-	+	-	+	-	+	+	-	+	+	+	-	+	-	+	+	+	-	-	+	17
RRIM 622	+	-	-	+	+	+	-	+	+	+	-	-	+	-	+	-	+	+	-	+	+	+	+	+	+	-	+	-	-	+	-	-	-	-	+	17
RRIM 623	+	-	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	-	+	-	+	-	+	+	-	-	+	+	+	+	+	+	+	21

Table I. contd.

Clone	Protein band number																																	Total		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33			
AV 385	+	-	-	+	-	+	-	+	+	+	-	-	+	-	+	+	-	+	+	-	-	+	-	+	-	+	-	+	-	-	-	-	-	+	16	
AV 427	+	-	+	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+	+	-	-	-	-	+	-	-	-	+	-	-	-	+	+	+	20	
AV 1734	+	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	-	+	+	-	+	-	-	-	-	-	21	
AV 2037	+	-	+	+	-	+	+	+	+	+	-	+	+	-	+	+	-	+	-	-	+	-	-	+	-	+	-	-	-	+	-	-	-	+	18	
PR 248	+	-	+	-	+	+	+	+	+	+	-	+	+	-	+	-	-	+	+	-	-	-	-	+	-	+	-	+	+	+	+	-	+	+	20	
PR 254	+	-	+	+	-	+	-	+	+	+	-	-	-	+	+	-	-	+	+	-	+	-	-	+	+	-	-	-	-	+	-	-	-	+	16	
TR 1542	+	-	+	+	-	+	-	+	+	+	-	+	-	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	+	-	-	+	-	+	19	
TR 3702	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-	+	-	-	+	+	-	+	-	+	-	+	+	-	-	-	-	+	21	
R 6	+	+	+	+	-	+	-	+	+	+	-	+	+	-	+	+	-	+	+	+	-	+	-	+	+	+	+	-	-	+	-	-	-	+	21	
IR 7	+	+	+	-	+	+	-	+	+	+	-	+	-	-	+	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	-	-	19	
IRCI 6	+	-	+	+	-	+	-	+	+	+	-	+	+	-	+	-	-	+	+	-	-	-	-	+	+	+	-	+	+	+	-	+	+	+	20	
GT 1	+	-	-	+	-	+	-	+	+	+	-	-	+	-	+	+	-	+	+	-	+	-	-	+	-	+	-	+	+	+	+	-	+	-	18	
WJ 1	+	-	+	+	+	+	-	+	+	+	-	+	+	-	+	+	-	+	+	-	-	-	-	+	-	-	+	+	+	+	-	+	-	+	20	
WR 101	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	-	+	+	+	+	+	+	+	26	
IAN 45/710	+	+	+	+	-	+	-	+	+	+	-	+	+	-	+	+	-	+	+	-	+	-	-	+	-	+	-	+	+	+	+	+	+	-	+	22
WG 6278	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+	25
GI 1	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	+	-	+	+	-	+	-	-	+	+	+	-	+	-	+	-	+	-	+	21	
<b>Total</b>	<b>72</b>	<b>11</b>	<b>50</b>	<b>48</b>	<b>31</b>	<b>72</b>	<b>23</b>	<b>69</b>	<b>72</b>	<b>72</b>	<b>5</b>	<b>36</b>	<b>54</b>	<b>15</b>	<b>72</b>	<b>53</b>	<b>10</b>	<b>71</b>	<b>58</b>	<b>19</b>	<b>45</b>	<b>39</b>	<b>4</b>	<b>72</b>	<b>18</b>	<b>61</b>	<b>19</b>	<b>33</b>	<b>50</b>	<b>36</b>	<b>26</b>	<b>17</b>	<b>51</b>			

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**Table 2.** Summary distribution of different protein bands in clones of respective categories

Category	Band number															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
I	P	NP	S	S	S	P	S	P	P	P	NP	S	S	S	P	S
II	P	S	S	S	S	P	S	P	P	P	S	S	S	S	P	S
III	P	S	S	S	S	P	S	P	P	P	NP	S	S	S	P	S
IV	P	S	S	S	S	P	S	P	P	P	S	S	P	S	P	S
V	P	NP	S	S	S	P	S	S	P	P	NP	S	S	S	P	S
VI	P	S	S	S	S	P	S	P	P	P	S	S	S	S	P	S
VII	P	S	S	S	S	P	NP	P	P	P	NP	S	S	NP	P	S
VIII	P	S	S	P	S	P	S	P	P	P	NP	S	P	S	P	P

**P** = Observed in all the clones of the category

**NP** = not observed in all the clones of the category

**S** = observed only in some of the clones of the category

patterns of different categories is given in Table 3. Protein bands No. 1, 6, 9, 10, 15 and 24 were present in all the zymograms. Band No. 11 was found only in 5 clones namely RRIC 7, RRIC 28, RRIC 60, RRIC 111, RRIC 114 and the band No. 23 was observed in 4 clones namely RRIC 103, RRIM 612, RRIM 622 and WG 6278. The highest number of protein bands was observed in the clone WR 101 where 26 bands were visible. The clones RRIC 51 and RRIC 129 exhibited 14 visible protein bands, the least number observed among the 72 clones investigated. More than 20 protein bands were observed in 31 clones. A fast moving protein band No. 33 was observed in 53 clones which had a Rf value close to bromophenol blue, the front marker in the gels (Table 1).

Table 3. Summary of important differences between protein patterns of different categories.

Category	Bands common to all the clones in a particular category	Bands not observed in all the clones in a particular category
I	8, 18	2, 11
II	8, 18	23
III	8, 18	11, 23
IV	8, 13, 18	23, 25
V	18, 26	2, 11
VI	8, 18	17, 23, 27
VII	8, 19, 21, 26	7, 11, 14, 17, 20, 25, 31
VIII	4, 8, 13, 16, 18	11, 17

(Note : Bands common to all the categories - 1, 6, 9, 10, 15, 24)

Protein bands No. 8, 9 and 10 were quite thin, sharp and distinct when compared to other bands, bands No. 9 and 10 were observed in all the clones investigated whilst band No. 8 was observed only in 69 clones. These bands were found to be quite sensitive to physiological changes of the tree (Prematillake 1981). They disappeared on Ethrel stimulation of the tree and the bands reappeared when the stimulation is over. These bands also disappeared under some disease conditions of the tree. For example, they were found to be absent in the latex of *Fomes* affected trees (Prematillake 1981). The intensities of these 3 bands were carefully studied and the observations are given in Table 4. Protein band No. 8 was not observed in 3 of the clones investigated, namely RRIC 52, RRIC 100 and RRIC 109. Of the three bands No. 9 was the most intense in almost all the clones. The intensity and sharpness of these 3 bands were very high in clones AV 427 and AV 2037.

Walujono and Suseno (1973) have obtained more protein bands at higher pH levels than at low pH levels and these protein patterns have been found to be less characteristic for clones. At low pH values they obtained a fewer protein bands with considerable differences among clones. They figured out a maximum of 13 bands at pH 4 in their typical diagram. No attempt was made in this study to compare the band patterns at low and high pH levels. Instead, it was attempted to categorize

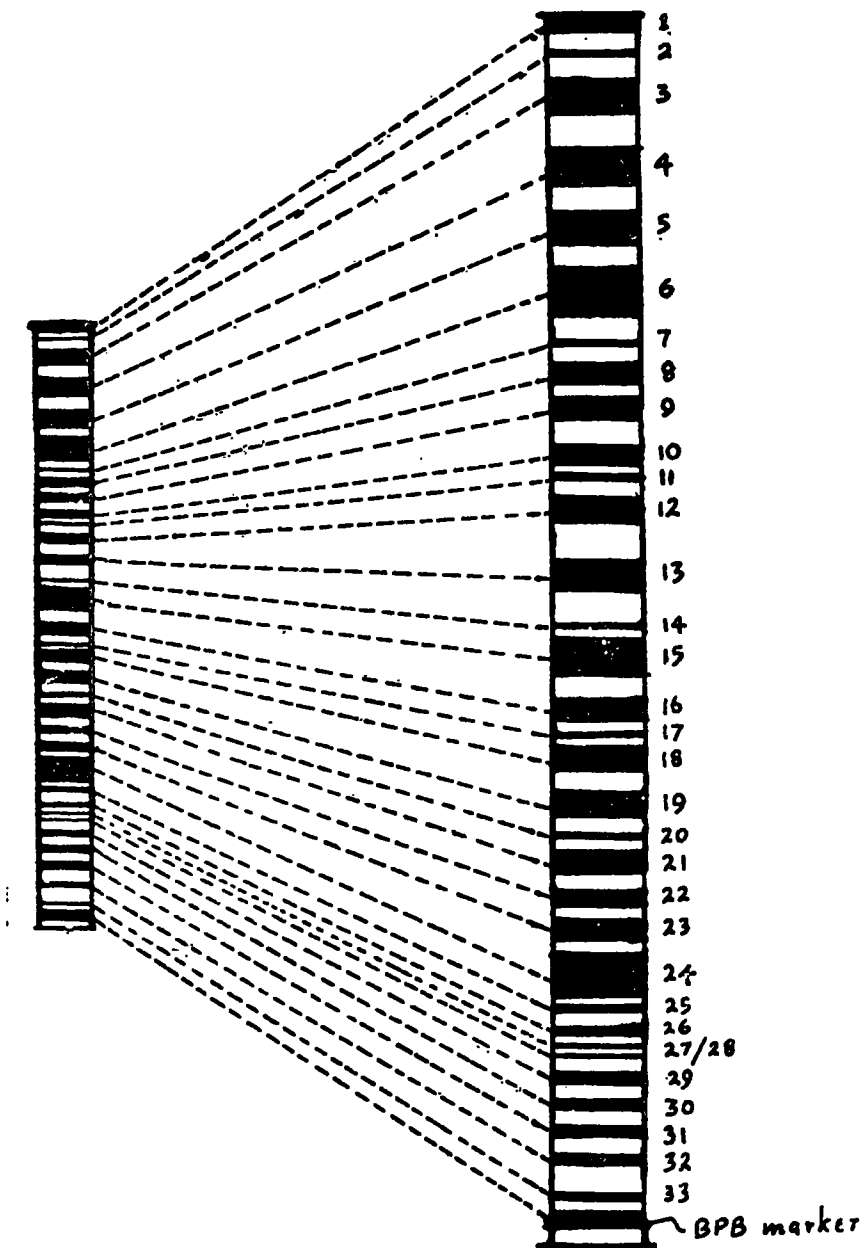


Fig. 1. Typical diagram of protein patterns obtained by PAGE electrophoresis of A-serum of 72 *Hevea* clones commercially planted in Sri Lanka.

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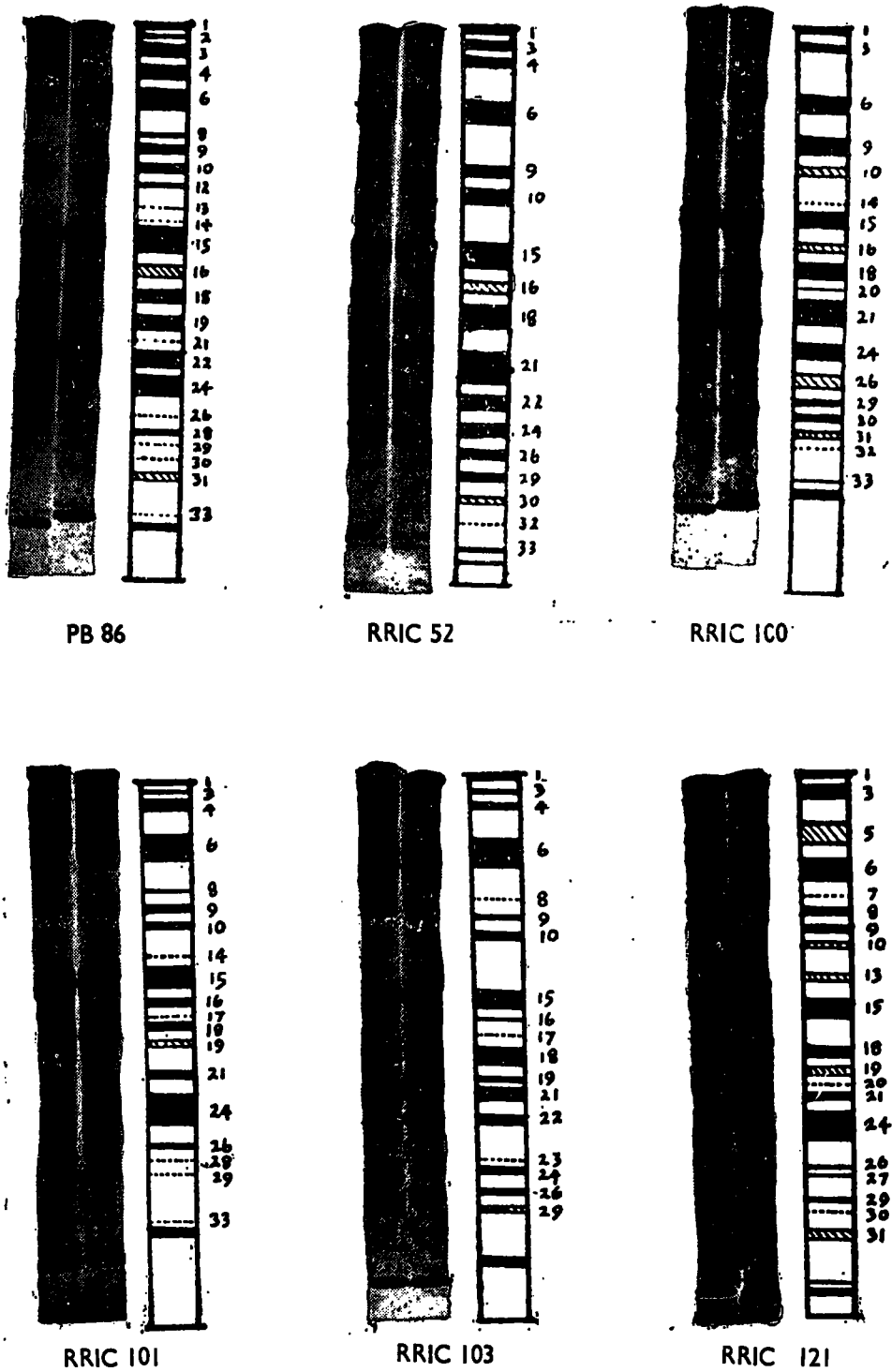


Fig. 2. A-serum protein patterns of 5 popular *Hevea* clones in Sri Lanka.

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Table 4 *Distribution and intensity of protein band numbers 8, 9 and 10 in different clones*

Clone	Band number			Clone	Band number		
	8	9	10		8	9	10
PB 86	++	++++	+++	RRIC 122	+	+++	++
RRIC 7	++	++++	+++	RRIC 123	+++	++	+
RRIC 22	++	+++	+++	RRIC 124	+	+++	+
RRIC 28	+++	+++	+++	RRIC 125	++++	++++	+++
RRIC 36	+++	+++++	+++	RRIC 126	+	+++	+++
RRIC 37	+	++++	++	RRIC 127	++	++	+
RRIC 39	+	+++	++	RRIC 128	++	+++	++
RRIC 41	+	+++	++	RRIC 129	++	++	++
RRIC 45	+	+++	+++	RRIC 130	++	++	++
RRIC 51	+	+++	+	RRIC 131	++	+++	++
RRIC 52	—	+++	+++	RRIM 513	+++	++++	+++
RRIC 54	++	++++	+++	RRIM 600	+	++++	+++
RRIC 55	++	++++	+++	RRIM 603	++++	++++	++
RRIC 60	++	++++	+++	RRIM 605	+	+++	++
RRIC 75	+++	++++	++	RRIM 607	+	+++	++
RRIC 76	+	+++	++	RRIM 612	++	++++	+++
RRIC 88	+	++	++	RRIM 622	++	++++	+++
RRIC 89	+	++++	+++	AV 385	+	++	+
RRIC 90	++	++++	+++	AV 427	+++++	++++	+++
RRIC 100	—	++++	++	AV 1734	++	++++	+++
RRIC 101	+	+++	++	AV 2037	+++++	+++++	+++
RRIC 102	++	++	+	PR 248	++	++++	+++
RRIC 103	+	++	++	PR 254	+	+++	++
RRIC 104	+	+++	+++	TR 1542	+	+++	++
RRIC 106	++	+++++	+++	TR 3702	+	+++	++
RRIC 107	++	+++	++	IR 6	+	++++	++
RRIC 108	+	++++	++	RRIM 623	+	++	++
RRIC 109	—	++	++	IR 7	+	+++	++
RRIC 110	++++	++	+	IRCI 2	+++	+++	+++
RRIC 111	++	+++++	+++	IRCI 6	+++	++++	+++
RRIC 112	+	++	++	GT 1	+	+++	++
RRIC 113	+	+++	++	WJ 1	+	+++	++
RRIC 114	++	++++	+++	WR 101	++	+++++	+++
RRIC 117	+++++	++	+++++	IAN 45/710	+	+++	++
RRIC 118	++	++	++	WG 6278	+++	++++	+++
RRIC 121	+++	++	+	GI 1	+++	++++	++++

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Table 5. *Classification of 72 Hevea clones into 8 main groups*

Group I		Group II		Group III		Group IV	
RRIC	22	PB	86	RRIC	36	RRIC	55
RRIC	39	RRIC	7	RRIC	106	RRIC	114
RRIC	45	RRIC	37	RRIC	107	RRIC	117
RRIC	54	RRIC	41	RRIC	108	RRIC	121
RRIC	76	RRIC	60	RRIC	113	RRIC	122
RRIC	101	RRIC	88	RRIC	123	GT	1
RRIC	126	RRIC	111	RRIC	131	Wj	1
RRIC	129	RRIC	125	RRIM	603	WR	101
RRIM	607	RRIC	127	RRIM	605		
RRIM	622	RRIC	128	IRCI	6		
RRIM	623	IR	7	GI	1		
AV	385						
AV	427						
AV	2037						
PR	248						
TR	1542						
Group V		Group VI		Group VII		Group VIII	
RRIC	52	RRIC	28	RRIC	51	RRIC	130
RRIC	100	RRIC	90	RRIC	75	RRIM	600
RRIC	102	RRIC	124	RRIC	89	TR	3702
RRIC	103	RRIM	513	RRIC	112	Wagga	6278
RRIC	104	PR	254	RRIM	612	AV	1734
RRIC	109	IRCI	2			IAN	45/710
RRIC	110	IR	6				
RRIC	118						

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and identify, wherever possible, different *Hevea* clones using their A-serum protein patterns at high pH levels: The maximum number of protein bands observed in the present investigation in a single clone was 26, in the clone WR 101.

The protein patterns of 72 different *Hevea* clones were categorized into 8 different groups based on the basic pattern of the zymograms (Table 5). These zymograms of clones in each category appear to have close resemblances although the differences could be noticed by careful observation. Of the clones investigated, protein patterns of only a few clones namely RRIC 52, AV 1734, RRIC 100 and Wagga 6278 were found to be distinctly characteristic compared to other clones.

Like in the study of Walujono and Suseno (1973), the present investigation also shows that the A-serum protein patterns can be used for identification of some of the clones, if not all or many, particularly those with characteristically distinct bands such as RRIC 52, AV 1734, RRIC 100 and Wagga 6278. The main drawback, of course is the close resemblances between the protein patterns of a large number of clones. This also necessitates great skill and experience for accurate identification of clones, posing some problems in practical application of this technique as a routine method for characterization of clones. The separation of proteins at a higher pH level seem to have contributed very little in improving the applicability of the technique as a routine procedure for clone identification just as in the study of Walujono & Suseno (1973). However this does not eliminate the possibility of using it as an accurate method for clone characterization by comparing with a protein map, pre-prepared accurately.

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