

## ACCUMULATION OF FREE PROLINE IN BARK AND LATEX OF *HEVEA BRASILIENSIS* AFFECTED BY BROWN BAST

By

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### SUMMARY

*Free proline accumulation in clone RRIC 101 of Hevea brasiliensis was investigated. The bark and the latex samples collected from both healthy and brown bast affected trees were analysed for free proline content. The average bark proline concentration/unit present in the brown bast trees was found to be considerably higher ( $P = 0.10$ ) than that of healthy trees. The accumulation of free proline in latex was found to be significant in brown bast trees.*

### INTRODUCTION

Brown bast or the bark dryness which is generally regarded as a physiological disorder of *Hevea* tree is of great economic importance since it leads to the loss of yield or latex particularly in vigorous high yielders. Despite considerable amount of research, the actual cause of this disorder is not yet known although it has been attributed to nutritional stress, and over exploitation. Some workers have also suggested that water stress could be responsible for the development of brown bast (Schweizer, 1936; Vollema, 1949; Compagnon, 1953). However this view has not been supported by experimental evidence. It has been reported that the free proline content in plants increases with water stress (Routley, 1966; Stewart, 1972; Waldren *et al*, 1974). Similar increase in free proline content of excised leaves of many agricultural crops under drought stress have also been reported (Routley, 1966; Barnett, 1966) and in water stressed lemon trees (Yoseph Levy, 1980). Proline which increases proportionately faster than other amino acids in plants under water stress has been suggested as a parameter to measure physiological dryness (Bates, 1973; Juhasz *et al*, 1971; Singh *et al*, 1972).

The objective of the present investigation was to see if there is any accumulation of free proline in *Hevea* under water stressed conditions and if so, its implications in relation to actual cause of this disorder. It was also aimed at the possibility of using any such accumulation of proline as an early warning signal for brown bast.

### MATERIALS AND METHODS

Clone RRIC 101 which is very susceptible to brown bast was selected for this study. A suitable area at Eladuwa State Plantation of Kalutara district in the low country wet zone was selected and a random sample of healthy (H) as well as brown bast (BB) affected trees was considered for sampling. Trees found in the experimental block were approximately of the same age and of the same size.

Determination of free proline content was based on the method described by Levy (1980). Each bark disc was cut into small pieces and placed in the vials containing 2 ml of 3% aq. 5 - Sulfosalysilic acid, frozen promptly in the field in a salt-ice mixture and kept

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in a freezer at  $-15^{\circ}\text{C}$  for at least 24 h. Free proline was extracted by shaking vials containing bark samples for 30 min at  $30^{\circ}\text{C}$  and filtering through Whatman No. 4 filter paper. One ml of extract was placed in 1 ml glacial acetic acid and 1 ml acid ninhydrin (2.5 g ninhydrin, 60 ml glacial acetic acid, 20 ml 6 M  $\text{H}_3\text{PO}_4$ ). Colour was developed for 1 h at  $100^{\circ}\text{C}$ . The reaction was terminated in an ice bath and the chromophore was extracted with 4 ml toluene. Absorbance was read at 520 nm after appropriate dilution with toluene and proline concentration was calculated per bark volume unit ( $\mu\text{mol cm}^{-3}$ ).

The preliminary trial was to study the difference in bark proline content between BB and H trees and to investigate the distribution pattern of proline content at different height levels around tapping panel. Twelve BB trees and 12 healthy trees found adjacent to those BB trees (AH) were randomly selected. An independent set of 6 healthy trees found far away from the brown bast trees (IH) was also selected randomly. Bark discs at different heights of the tapping panel side e.g. 10, 50, 70, 125, 150 and 200 cm from the stock-scion union were punched in the field using a bark borer. The diameter of the bark borer was 2 cm. Thickness of each bark sample was measured.

To study the proline levels in latex of brown bast and healthy trees and also to see whether the proline concentration of tapping panel side is different from that of the opposite side, a total of 38 trees were sampled (15 BB ; 15 AH ; and 8 IH). One bark sample from the centre of the tapping panel, 10 cm below the tapping cut and the other from just the opposite side at the same height were collected for analysis. One latex sample from each tree was collected in a vial immersed in ice and kept in a deep freezer for 3 weeks. F-serum (0.05 ml) was used for proline determination. The proline concentration of latex was calculated in  $\mu\text{mol ml}^{-1}$ .

Results were analysed statistically using paired t test to compare BB and AH trees and independent t test to compare BB and IH trees.

## RESULTS

A preliminary trial to study the effect of induced water stress in young *Hevea* plants on proline accumulation was carried out in the green house. Leaf samples from control and water-stressed plants were collected as described above and were tested for proline content. A marked increase in the proline content of leaves of water stressed plants was observed compared to control plants which were watered as usual. The rest of this study was planned based on this observation that there is an accumulation of proline in *Hevea* plants, under water stressed conditions.

### Proline content at different height levels of BB and AH trees

Mean proline concentration at each height of BB trees was found to be considerably higher than that of AH trees except for two heights i.e. 70 cm and 125 cm (Table 1). But this difference was not significant. Highest proline concentration was observed at 125 cm.

Table 1. *Proline concentration in brown bast (BB) and adjacent healthy trees (AH)*

Height (cm) measured upwards from stock/scion union	Mean proline concentration ( $\mu$ mol cm <sup>-3</sup> )		Difference	Paired 't' test t-values d.f. 11
	Brown bast	Adjacent healthy		
10	0.359	0.232	0.127 <sup>NS</sup>	1.514
50	0.344	0.259	0.085 <sup>NS</sup>	1.353
70	0.381	0.397	-0.016 <sup>NS</sup>	0.178
125	0.405	0.440	-0.035 <sup>NS</sup>	1.569
150	0.395	0.385	0.010 <sup>NS</sup>	1.025
200	0.339	0.268	0.071 <sup>NS</sup>	0.779
Mean	0.370	0.330	0.040 <sup>NS</sup>	1.021

NS : Not significant at 5% level.

**Proline content in bark tissue of BB and IH trees**

The proline content of the bark tissue of BB and IH trees at different heights is given in Table 2. Bark proline concentration at each height of BB trees was found to be considerably higher than that at corresponding height of IH trees. But this difference at each height was not significant. Mean proline concentration of overall heights of BB trees was significant (at 5% level) than that of IH trees. Proline concentration at heights 70 cm and 125 cm was relatively high when compared to the other heights (Table 2).

Table 2. *Bark proline concentration in brown bast (BB) and independent healthy trees (IH)*

Height (cm) measured upwards from stock/ scion union	Mean proline concentration ( $\mu$ mol cm <sup>-3</sup> )		Difference	Independent 't' test (t - values) d.f. 16
	Brown bast	Independent healthy		
10	0.359	0.230	0.139 <sup>NS</sup>	1.015
50	0.344	0.221	0.123 <sup>NS</sup>	1.637
70	0.381	0.342	0.039 <sup>NS</sup>	0.482
125	0.405	0.310	0.095 <sup>NS</sup>	1.236
150	0.395	0.327	0.068 <sup>NS</sup>	1.226
200	0.339	0.179	0.160 <sup>NS</sup>	1.762
Mean	0.370	0.268	0.104*	2.342

NS : Not significant at 5% level

\* : Significant at 5% level

**Proline content in latex and bark tissue of BB and IH trees**

Proline content in latex of BB, AH and IH trees and mean bark proline concentration of BB, AH and IH trees on the tapping panel side and opposite side are given in Table 3. Mean bark proline concentration of BB trees was considerably higher than that of both AH and IH trees. But the difference was not significant when BB and AH trees were compared. It was significant at 100% level when BB and IH trees were compared. Mean latex proline concentration of BB trees was significant (5% level) when both BB vs AH and BB vs IH trees were compared (Table 3).

Table 3. *Bark and latex proline concentration in brown bast (BB), adjacent healthy (AH) and independent (IH) healthy trees*

Sample	Mean proline concentration		
	Bark (tapping panel side) $\mu$ mol cm <sup>-2</sup>	Bark (opposite side) $\mu$ mol cm <sup>-2</sup>	latex $\mu$ mol m <sup>-1</sup>
1. Brown bast	0.1189	0.0895	0.8158
2. Healthy adjacent	0.0866	0.1042	0.5737
3. Healthy independent	0.0772	0.0474	0.5023
Difference (1) — (2)	0.0323 <sup>NS</sup>	0.0147 <sup>NS</sup>	0.2421*
( $\pm$ S.E.)	( $\pm$ 0.0183)	( $\pm$ 0.0251)	( $\pm$ 0.0650)
Difference (1) — (3)	0.0417	0.0421 <sup>NS</sup>	0.3135*
( $\pm$ S.E.)	( $\pm$ 0.0237)	( $\pm$ 0.0248)	( $\pm$ 0.0916)

NS : Not significant at 5% level

\* : Significant at 5% level

a : Significant at 10% level.

**Comparison of bark proline content in BB, AH and IH trees**

A comparison of proline concentration in the bark tissue of BB, AH and IH trees, on the tapping panel area and the opposite side is given in Table 4. The difference of proline concentration between tapping panel side and the opposite side of an individual tree was not significant when BB and AH trees were considered. This difference was significant at 5% level, when the two sides of IH trees were compared.

Table 4. Bark proline concentration on the tapping panel side and the opposite side

	Mean proline concentration		Difference (+ S.E.)
	Tapping panel side $\mu$ mol cm <sup>-3</sup>	Opposite side $\mu$ mol cm <sup>-3</sup>	
Brown bast	0.1189	0.0895	0.0294 <sup>NS</sup> (+ 0.0179)
Healthy adjacent	0.0866	0.1042	0.0176 <sup>NS</sup> (+ 0.012)
Healthy independent	0.0772	0.0474	0.0298* (+ 0.0054)

NS : Not significant at 5% level

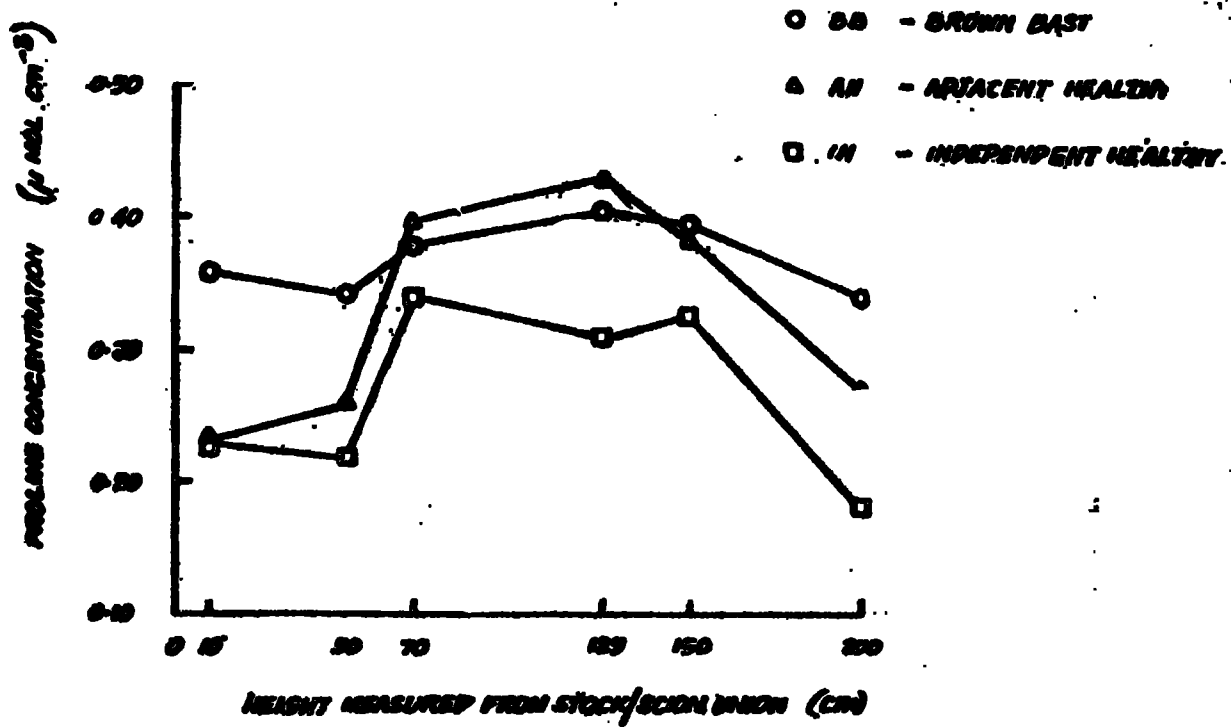
\* : Significant at 5% level

#### DISCUSSION

Free proline concentration in water stressed plants was first reported by Kemble *et al* in 1954. Since then, free proline accumulation has been reported for many, mostly annuals, plant species including horticultural crops like apple, loquat, pea, Jerusalem artichoke, pumpkin, carrot, phaseolous beans, citrus and other various ornamentals (Dashea *et al*, 1981). In our study too a greater accumulation of free proline in bark and latex samples of brown bast affected *Hevea* trees, as compared to healthy *Hevea* trees located at a distance from affected trees was observed. This suggests that the water stress situation is associated with brown bast although it is not known whether it is the cause or a secondary effect of brown bast.

In the preliminary trial where different heights along the tapping panel were concerned, it was observed that the bark proline concentration was higher around the area closer to the tapping cut, ranging from 70 — 125 cm from the stock-scion union as shown in Fig. 1. This area generally fell in between 5 — 15 cm below the tapping cut. Based on this, the bark samples for the second experiment were taken from 10 cm below the tapping cut. Paranjothy *et al* (1975) have also reported that the majority of brown bast affected trees observed in their study, showed the disorder in an area closer to the tapping cut.

The mean proline concentration of overall heights of bark of BB trees was found to be significant when compared to that of independent healthy (IH) trees (Table 2). This difference was not significant when BB and AH trees were compared (Table 1). Similar observations were made in experimental II too, as shown in Table 3. The mean total proline concentration of bark of the tapping panel side was considerably higher when compared to the opposite side, only in the case of IH trees (table 4). This clear difference of proline concentration of IH vs. BB and not between AH vs. BB may be due to that most of the AH trees may already be in the process of being affected by brown bast making them really stand in between BB and H. The findings of Zoysa *et al* (1983) reveals that



**FIG.1. DISTRIBUTION PATTERN OF PROLINE CONTENT AT DIFFERENT HEIGHTS ALONG THE TAPPING PANEL**

the clone RRIC 101, often shows a clustered pattern in distribution of brown bast trees in a particular field. This also points to the possibility that AH trees are more liable to be affected by brown bast than IH trees which are in a slightly different environment with respect to soil moisture level etc.

When the proline concentration of latex was investigated, it was found that the free proline concentration in latex of BB trees was significantly higher (5% level) than that of both AH and IH trees. Therefore the use of latex proline level as an early warning signal for BB could be more indicative than that of bark, as the amino acids in latex seem to be more sensitive, even to minute biochemical changes. This also shows the importance of studying physiological and biochemical changes in latex, in finding the cause for this physiological disorder in *Hevea*.

However, the possible use of proline as the sole parameter to measure water stress may not always be successful. It has been observed that proline does not accumulate until the water stress is quite severe (Mc Michael *et al*, 1977 ; Sairam and Dube, 1984). Hence free proline may not be a good indicator of the onset of plant water stress. The trees tested in this study for proline accumulation were affected by BB, quite severely. It might be useful to study the extent of proline accumulation in latex of trees affected by BB at varying degrees. One could use free proline accumulation in investigations aimed at finding whether water stress is involved in a particular situation.

Climatic conditions, particularly soil moisture levels also seem to play a role in BB condition and a closer look at this seems worthwhile especially in view of the different levels of proline accumulation in IH and AH trees. The clone RRIC 101 used in this study seems to show various responses towards brown bast condition when it is grown in different climatic regions. It has been reported that this particular clone is more resistant to brown bast when it is planted in intermediate and dry zones like Matale, Moneragala, Bible etc (Fernando, *et al* 1983). It is generally known that in dry zones, when high yielders are tapped, the amount of latex dripped from the tree per day is less because of faster drying of the tapping cut due to relatively high temperature, high wind velocity and so on. But when the same clone is tapped in wet zones like Kalutara, where the same climatic factors are different, (*i.e.* relatively low temperature, low wind velocity) the latex seems to drip for hours. As a result the high yielders such as RRIC 101 planted in wet zones under go much stress especially in relation to water, as it is the main constituent of latex. Interestingly, in our experimental block, which was a topographically slopy area, we observed that most of the BB trees with a high proline content in both bark and latex were found at the top of the slope and most of the independent healthy trees which had low proline concentrations both in bark and latex were found at the bottom where streams and rice fields are located. This points to a possible involvement of soil moisture content at the onset of brown bast, specially when clonal and climatic factors are considered. The high proline levels in latex and bark of BB trees and the low proline levels of IH trees close to the high moisture area, also support this view.

Although there seems to be a fairly, clear involvement of a water stress situation in the BB trees investigated in this study, which is further supported by an accumulation of proline in BB trees, it is not known whether water stress situation is actually the cause of

this disorder. Further investigations into both water stress and soil moisture content, along with other physiological and nutritional parameters are necessary before arriving at any firm conclusions.

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