Rubber Tree Cultivation and Improvement: Laticifer Ring Count and Latex Yield Assessment of Rubber Species

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Abstract— *Rubber trees (Hevea spp.), the wilderness trees originating from rain forests of the Amazon, have been domesticated outside South America for more than a century. The trees, specifically of the species Hevea brasiliensis, are now widely established in the tropics especially in Southeast Asia. Nevertheless, opportunities to improve the latex yield productivity of these cultivated rubber trees are limited by their narrow genetic base since they have been descended from a small selection of seedlings derived from seeds collected in the 1800's. Commercial rubber trees hence face this genetic vulnerability of inbreeding depression that could hamper progress in crop improvement. To explore the feasibility of broadening the genetic base, various Hevea species, viz. Hevea brasiliensis, Hevea benthamiana, Hevea camargoana, Hevea guianensis, Hevea nitida, Hevea pauciflora, Hevea rigidifolia and Hevea spruceana, were assessed for their possible contribution to the genetic improvement of the cultivated rubber trees particularly in latex yield output. The assessment showed that Hevea benthamiana, and Hevea spruceana to be promising in terms of the number of laticifer rings in the bark and latex yield. They are promising candidates for incorporation into the improvement programmes of the rubber tree in Malaysia.*

Keywords— Hevea species, latex yield, laticifer rings.

I. INTRODUCTION

Key inventions in the 1820s introduced novel uses for natural rubber, Charles Macintosh produced the earliest water-resistant rubberised fabrics while Thomas Hancock invented the rubber masticator that could cut and compressed rubber into moulded solid rubber, Hancock further improved the application of natural rubber latex in various surgical tools, water resistant clothing, footwear, hoses, rubber belts, engine components and rubber inflatables (Barlow, 1978; MRB, 2005; MRB, 2009; Priyadashan, 2011). The demand for natural rubber reached new heights following the introduction of vulcanization by Charles Goodyear in 1839, a process where rubber is heated in the presence of sulphur. This stabilises the rubber at both high and low temperatures while being resistant to melting and able to retain its elasticity, such characteristics being requisite for rubber tyres in the automobile industry. The invention of the motorcar and pneumatic tyres in the mid-1800s was an important factor that triggered higher demand for natural rubber latex as a raw material worldwide (Barlow, 1978; Baulkwill, 1989). The unique properties of rubber and its versatility have resulted in its use in a wide assortment of products.

The demand for rubber latex has led to extensive cultivation of *Hevea brasiliensis* not only in Asia (Malaysia, Thailand, Indonesia, India, Myanmar, the Philippines, Cambodia, Vietnam, China and Sri Lanka) but also in Africa (Nigeria, Cameroon and Ivory Coast). In Malaysia, the natural rubber industry caters to most of the livelihoods of smallholding growers, which is made up of mostly senior residents, who live off the cultivated rubber trees many decades ago.

Latex is produced in laticifers which are cells fused end to end to form continuous vessels. Laticifers are found in all parts of the rubber tree although tapped latex is derived mainly from those in the bark of the trunk. In cross-section under the microscope, laticifers in the bark appear as concentric rings surrounding the trunk. The roles of latex in rubber trees and other plant species have been suggested by Bealing (1965), Hunter (1994), Rudall (1994), Agrawal and Konno (2009), and Kajii *et al*. (2014) to be highly associated with: (1) protection against injury; (2) storage of carbon and its derivatives; and (3) storage of water and the regulation of its supply. Various rubber species, viz. *Hevea brasiliensis, Hevea benthamiana, Hevea* *camargoana, Hevea guianensis, Hevea nitida, Hevea pauciflora, Hevea rigidifolia* and *Hevea spruceana* available in Malaysia (Schultes, 1990). However, these rubber species were not fully utilized in the recent rubber improvement programmes in the country. Thus, these rubber species were assessed for their possible contribution to the improvement of the cultivated rubber trees in latex yield output.

II. MATERIALS AND METHODS

2.1 Laticifer Ring Count

Bark samples from eight different *Hevea* species, viz. *Hevea brasiliensis, Hevea benthamiana, Hevea camargoana, Hevea guianensis, Hevea nitida, Hevea pauciflora, Hevea rigidifolia* and *Hevea spruceana,* were collected from the tree trunk at a height of 1.5 m from ground level, using a bark borer that penetrated at least 6 mm into the bark layer. After the samples were collected, the holes made by the bark borer were covered with Shell petroleum jelly healing compound to assist healing of the wounded bark and allow subsequent bark renewal. In the laboratory, free-hand sectioning was carried out to examine and compare the bark morphological characteristics of the eight *Hevea* species. Ten bark samples of each species were softened with distilled water before cutting free-hand longitudinal sections of about 10 µm thickness using a sharp razor blade. The sectioned samples were stained with Sudan III and mounted on a glass slide for examination under an Olympus BH2 Light Microscope. The number of laticifer rings (laticifers) in the samples were counted and averaged according to different rubber species. Comparisons of means, standard errors and standard deviations were carried out. Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were also performed.

2.2 Latex Yield

A tapping cut was made across half the circumference of the tree trunk (1/2 S) by thin shredding of bark from the sloping cut at an angle of 30°, starting from the upper left and ending to the lower right of the tree trunk. Exuded latex was collected in a 100 ml test tube that attached to each sampled tree. Then, these collected samples were taken out from the test tubes and air-dried for 14 days over wooden racks. Latex yield from the tree was calculated as dry rubber weight and expressed as grams per tree per tapping (gtt). The yield for each species was subjected to comparison of means, standard errors and standard deviations. Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were also performed.

III. RESULTS

3.1 Laticifer Ring Count

The number of laticifer rings in eight *Hevea* species was analysed by a comparison of means, and by an Analysis of Variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) (Tables 1 and 2). The ANOVA analysis revealed significant differences between the mean number of laticifer rings between the species compared [$F(7, 72) = 13.797$, $p < 0.05$]. *H. brasiliensis* showed the highest mean number of rings at 14.3, followed by *H. benthamiana* at 12.0 and *H. spruceana* at 11.3. On the other hand, *H. camargoana and H. guianensis* had the lowest means among the species, at 6.7 and 3.5 respectively. DMRT analysis indicated that the mean laticifer ring count of *H. brasiliensis* was significantly higher than for the other species, whereas the count for *H. guianensis* was the lowest.

TABLE 1
MEAN, STANDARD ERROR AND STANDARD DEVIATION OF NUMBER OF LATICIFER RINGS IN THE BARK OF EIGHT *HEVEA* SPECIES.

Species	Mean	Standard Error	Standard Deviation
Hevea benthamiana	12.0 ab	1.125	3.559
Hevea brasiliensis	14.3 a	1.317	4.165
Hevea camargoana	6.7 d	0.761	2.406
Hevea guianensis	3.5 e	0.269	0.850
Hevea nitida	9.9 bc	0.706	2.234
Hevea pauciflora	9.4 bcd	0.718	2.271
Hevea rigidifolia	7.5 cd	0.778	2.461
Hevea spruceana	11.3 b	1.174	3.713

**** Means with the same letter are not significant different by DMRT, p<0.05.***

TABLE 2
ANOVA FOR NUMBER OF LATICIFER RINGS IN THE BARK OF EIGHT *HEVEA* SPECIES.

Source of Variation	*df*	*Sum of squares*	*Mean square*	*F*	*P*-value
Between groups	7	802.950	114.707	13.797	*0.000
Within groups	72	598.600	8.314		
Total	79	1401.550			

P-value: < 0.0005

3.2 Latex Yield

Analysis of Variance (ANOVA) analysis revealed significant differences [$F(7, 72) = 117.612$, $p < 0.05$] between mean latex yield of rubber species (dry rubber weight) was expressed as g per tree per tapping (gtt) from different *Hevea* species (Table 3 and 4). The results showed that *H. brasiliensis* produced the highest mean yield at 39.7 gtt, followed by *H. benthamiana* and *H. spruceana* with means at 24.7 gtt and 22.8 gtt respectively. *H. guianensis* showed the lowest mean yield at 4.1 gtt. DMRT analysis indicated that the mean yield for *H. brasiliensis* was significantly higher than those for all of the other species. Meanwhile, latex yield from *H. guianensis* was significantly the lowest among the eight rubber species.

TABLE 3
MEAN, STANDARD ERROR AND STANDARD DEVIATION OF LATEX YIELD (gtt) IN EIGHT *HEVEA* SPECIES.

Species	Mean	Standard Error	Standard Deviation
Hevea benthamiana	24.7 b	1.065	3.368
Hevea brasiliensis	39.7 a	1.535	4.855
Hevea camargoana	11.0 d	0.894	2.828
Hevea guianensis	4.1 e	0.379	1.197
Hevea nitida	17.3 c	0.775	2.452
Hevea pauciflora	17.1 c	1.320	4.175
Hevea rigidifolia	9.7 d	0.633	2.003
Hevea spruceana	22.8 b	1.041	3.293

Means with the same letter are not significant different by DMRT, p<0.05.

TABLE 4
ANOVA FOR LATEX YIELD (gtt) IN EIGHT *HEVEA* SPECIES

Source of Variation	*df*	*Sum of squares*	*Mean square*	*F*	*P*-value
Between groups	7	8505.00	1215.000	117.612	*0.000
Within groups	72	743.80	10.331		
Total	79	9248.80			

P-value: < 0.0005

3.3 Relationship between Number of Laticifer Ring Count and Latex Yield

Pearson's correlation coefficients were calculated to determine the relationships between the number of laticifer rings and latex yield among the eight *Hevea* species. The result showed a positive correlation between number of laticifer rings and latex yield with a correlation coefficient (r) = 0.698, which was statistically significant ($n = 80$, $p < 0.01$) as showed in Table 5.

TABLE 5
PEARSON'S CORRELATION COEFFICIENT BETWEEN NUMBER OF LATICIFER AND LATEX YIELD EXPRESSED IN GRAMME PER TREE PER TAPPING (gtt)

		Number of laticifer	Latex yield (gtt)
Number of laticifer rings	Pearson's correlation	1	0.698*
	Number of sample (N)	80	80
Latex yield (gtt)	Pearson's correlation	0.698*	1
	Number of sample (N)	80	80

**** Correlation coefficient at p<0.01.***

IV. CONCLUSION

H. brasiliensis, *H. benthamiana,* and *H. spruceana* showed the highest number of laticifers rings in the bark whereas *H. guianensis* had the lowest laticifer ring count among the rubber species studied. The number of laticifer rings were strongly correlated with latex yield produced in these *Hevea* species. Pearson's Correlation Coefficient indicated that increasing number of laticifer rings would have a tendency to accompany by increasing of latex yield. More attention should, therefore, be paid to laticifer ring count in future rubber improvement programmes. However, this characteristic is not a suitable criterion for the early selection in the nursery for young rubber plants less than 18 months old. In young plants, the laticifers are not fully developed and latex yield at this stage can give misleading results. In a nutshell, *H. benthamiana* and *H. spruceana* showed promising in term of number of laticifer rings and latex yield (dry rubber weight), apart from *H. brasiliensis*, to be considered for incorporation into the rubber improvement programmes in Malaysia.

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