CLONAL VARIATION IN QUANTITATIVE TRAITS OF LATICIFERS IN *HEVEA BRASILIENSIS*

Philipose Omman* and C.P. Reghu

*Department of Botany, Catholicate College, Pathanamthitta- 689 645, Kerala, India
Rubber Research Institute of India, Rubber Board, Kottayam-686 009, Kerala, India

Received: 12 July 2013 Accepted: 10 September 2013

Omman, P. and Reghu, C.P. (2013). Clonal variation in quantitative traits of laticifers in *Hevea brasiliensis*. *Rubber Science*, **26**(2): 279-289.

Latex vessels form a very imperative tissue system in the secondary phloem of *Hevea brasiliensis*. They run in the longitudinal axis of the stem with a specific angle of inclination for a particular *Hevea* clone. Rubber particles are synthesized in the laticiferous system present in the bark tissue and exploited through controlled wounding of the bark called tapping. Most of the laticifer characters have great significance for latex yield in *Hevea*. A detailed investigation on the latex vessel characters of ten clones has been made in the present study. The secondary phloem consisted of soft bark (SB) region devoid of stone cells and inner hard bark (IHB) region with stone cells. Number of latex vessel rows in both the location was higher in PB clones. The distance between laticifer rows exhibited significant clonal variability. About 90 per cent of the latex vessels were running contiguous to phloic rays and only 10 per cent remained non-contiguous to phloic rays. The articulated and anastomosing nature of laticifers were well supported by inter-connections and it was the highest in the clone RRII 105. Significant superiority of PB clones with respect to latex vessel diameter and total cross sectional area of laticifers was also noticed.

Keywords: Latex vessel diameter, Latex vessel rows, Laticifers, Laticifer area index

INTRODUCTION

Latex vessels or laticifers are cylindrical tubes distributed in the form of rows or rings in the secondary phloem and running in the longitudinal axis of the stem with specific angle of inclination (Omman and Reghu, 2008). Laticiferous system has been considered as the site of rubber synthesis in *H. brasiliensis* (Gomez, 1966; Southorn, 1966) and can be well stained by Oil Red O (Omman and Reghu, 2003). The number of laticifer rows has been reported as a quantitative anatomical parameter pertaining to latex yield in *H. brasiliensis* (Bobilioff, 1923; Gomez, 1966). The correlation of this trait with yield in *Hevea* has already been proved (Narayanan *et al.*, 1973; Narayanan *et al.*, 1974). The number of laticifer rows has been identified as a clonal character (Vischer 1921; Sanderson and Sutcliffe, 1929; Gottardi *et al.*, 1995) which varies considerably with tree age (Bryce and Campbell, 1917; Gomez *et al.*, 1972) and height (Sanderson and Sutcliffe, 1929; Gomez *et al.*, 1972) of the tree whereas the variability is not significant at young stages (Costa *et al.*, 2000).

The distance between laticifer rows has been considered as a yield contributing

Correspondence: C.P. Reghu (Email: reghu@rubberboard.org.in)

character in *Hevea* (Paiva *et al*., 1982) and considerable variation existed in different clones (Gomez *et al*., 1972; Goncalves *et al*., 1995). The latex vessels within a row are interconnected and also exhibited considerable variation (Premakumari *et al*., 1984; 1991). The diameter of the latex vessels determines the latex yield (Ashplant, 1928) and much variation has been recorded by various researchers (Gomez *et al*., 1972; Ho *et al*., 1973; Narayanan *et al*., 1973; Ho, 1972; 1976; Sethuraj, 1981; Premakumari and Panikkar, 1989). Similarly, laticifer area index has been used as a measure pertaining to tapping (Gomez *et al*., 1972) and yield in *Hevea* (Premakumari *et al*., 1993). Considering the importance of laticiferous system in the bark of *H. brasiliensis* the present study has been undertaken to understand the clonal variability in various quantitative traits of laticifers.

MATERIALS AND METHODS

Ten domesticated clones (Wickham base) of *H. brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. were selected from the Germplasm gardens I, II and III, at the Central Experimental Station of Rubber Research Institute of India, Chethackal, Ranni, Kerala. The gardens were laid out in randomised block design (RBD) with three replicates and three trees per plot. Ten clones *viz*., Tjir 1, Gl 1, PB 86, GT 1, PB 28/59, RRII 105, RRIM 600, RRIM 703, PB 235 and RRII 300 were selected for this study at an age of 17-21 years. Virgin bark samples were collected from mature trees of each clone at 150 cm height from the ground. The samples were fixed in formalin-acetic-alcohol (FAA) and standard procedures were adopted for microtomy and microscopy. Sections were taken at 5-10 μm thickness at different planes *viz*., cross sectional (CS), tangential longitudinal (TLS) and radial longitudinal (RLS) plane, and stained with Oil Red O (Omman and Reghu, 2003).

For the brevity of description and observations, the sections of the bark samples were divided into two parts *viz*., the soft bark zone (SB) contiguous to cambial region and the inner hard bark zone (IHB) immediately above the soft bark region. The parameters studied were (i) number of latex vessels in the SB region, (ii) number of latex vessels in the IHB region (iii) total number of latex vessel rows (iv) density of latex vessels (per row per 1mm distance) contiguous to phloic rays (v) density of latex vessels (per row per 1mm distance) non-contiguous to phloic rays (vi) diameter of latex vessels (μm) (vii) frequency of inter connections between latex vessels (viii) inter-distance between latex vessel rows (mm) in SB (ix) inter-distance between latex vessel rows (mm) in IHB (x) distance between cambial zone and first row of latex vessels (mm) and (xi) cross-sectional area of laticifers (laticifer area index).

Frequency of interconnections between laticifers was calculated per unit area ($5x10^{-2}$ mm^2) and the total cross sectional area of the latex vessels at a given CS of the bark (Laticifer area index) was computed as per Gomez *et al*. (1972).

For statistical analysis, Coefficient of Variation (CV) was calculated to ascertain the tree-to-tree variation within clones. Mean values were pooled to find out the CV values. Analysis of variation (ANOVA) was estimated to measure the extend of clonal variation between clones. Statistical analysis was carried out with MS Excel. Photomicrographs were taken in Leitz aristoplan research microscope attached to Wild MPS 46 photo automat using Kodak gold 35 mm colour film. Quantitative image analysis was done using Leica Q Win V.2.1 image analysis software.

RESULT AND DISCUSSION

Number of latex vessel rows in soft bark (SB) and inner hard bark (IHB)

In the longitudinal radial plane, latex vessels appeared as tubular structures in different rows. The number of latex vessel rows in SB region (Table 1) was the highest (20.06) in PB 28/59 (Fig. 1 a) and the lowest (6.89) in PB 86 (Fig. 1 b). Within clones, the tree-to-tree variation was high in GT 1 and RRII 105 and medium in rest of the clones. ANOVA indicated that PB 28/59 was statistically superior to RRIM 600, RRIM 703, RRII 300, GT 1 and PB 86; RRII 105 was superior to RRIM 703, RRII 300, GT 1 and PB 86. PB 235 was statistically superior to GT 1 and PB 86 (Table 1).

The number of LV rows in the IHB (Table 1) was maximum (25.78) in PB 86 (Fig. 1c) and minimum (9.67) in RRII 300 (Fig. 1d). The number of LV rows varied between trees in all the clones and variation was considerably high in RRII 300 and PB 28/59. The variation was medium in PB 235, GT1, RRII 105, Gl 1 and RRIM 600. Analysis of variation (Table 1) indicated that the clones PB 86, PB 235 and RRIM 703 were significantly superior to RRIM 600 and RRII 300. GT 1, RRII 105 and Gl 1 were superior to RRII 300. Latex vessels are mainly concentrated in the SB and IHB, of which 40 per cent in the former and 60 per cent in the latter. Similar findings were also made by Bobilioff (1920), Bryce and Gadd (1923), Sanderson and Sutcliffe (1929) and Gomez *et al.* (1972). In this context, drastic reduction in the number of laticifer rows in the SB of all clones as observed in the present study, may adversely affect the yield producing capacity, unless the latex vessel rows present in the IHB contribute considerable yield in *Hevea*.

Distance between laticifer rows in SB and IHB

Earlier workers have reported that the distance between laticifer rows is an important parameter in *Hevea* (Paiva *et al.*, 1982) and the average distance between consecutive rows of laticifers showed significant variation (Gomez *et al.*, 1972; Goncalves *et al.*, 1995). The present investigation also confirmed significant clonal variability for this trait. The average inter-row distance between laticifers in both SB and IHB regions is shown in Table 1. The inter-row distance between laticifers ranged from 0.07 mm to 0.13 mm in the SB region. The maximum distance (0.13 mm) was observed in PB 86 (Fig. 1 f) and the minimum (0.07 mm) in PB 28/59 (Fig. 1e). Within clones, tree-to-tree variation was high in RRIM 703 and RRII 300 and medium in PB 86, GT 1, RRIM 600 and PB 235. The remaining four clones *viz.*, Gl 1, PB 28/59, RRII 105 and Tjir 1 showed very low tree-to-tree variation. However, the clonal variability was not statistically significant for this trait.

The distance between adjacent laticifer rows in IHB was maximum in RRIM 600 and Tjir 1 (0.13 mm) and minimum in RRII 105 (0.07 mm). Within clones the trees exhibited medium variation in two clones *viz*. RRIM 600 and Gl 1 and the rest of them displayed low tree-to-tree variation. ANOVA (Table 1) revealed that RRIM 600, Tjir 1 and RRII 300 are superior to PB 28/59, GT 1, Gl 1, RRIM 703 and RRII 105. Similarly PB 235 and PB 86 also showed superiority over RRIM 703 and RRII 105. Majority of the clones showed high number of latex vessel rows with less inter-row distance in both SB and IHB. This may facilitate to accommodate more number of latex vessel rows in the SB zone as reported by Narayanan *et al.* (1974). Though the number of laticifer rows varied in SB and

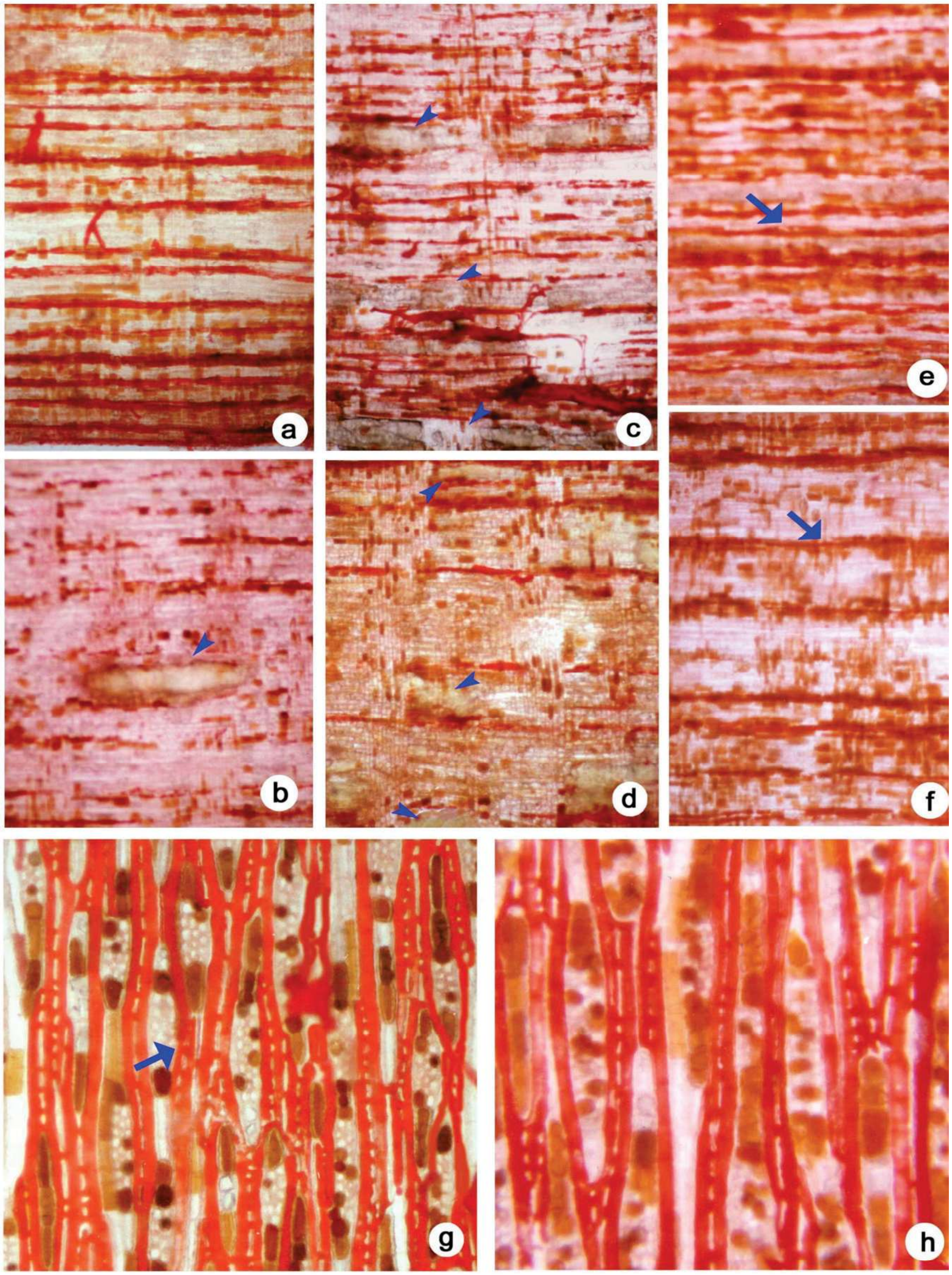

Fig. 1. a-h. Bark sections stained with Oil Red O. a- PB 28/59 with maximum number of laticifer rows in soft bark. b- PB 86 with minimum laticifers rows in soft bark. c- PB 86 with maximum laticifer rows in inner hard bark. d- RRII 300 with minimum laticifer rows in inner hard bark. Stone cell at arrows. e- PB 86 with minimum laticifer inter-row distance in SB. f- PB 28/59 maximum laticifer inter-row distance in SB. g- GT 1 maximum density of latex vessels contiguous to rays (note arrow) . h-PB 235 minimum density of latex vessels contiguous to rays. a-g RLS, X 75

Table 1. **Number and distance between laticifer rows in soft bark and inner hard bark and distance from cambium to 1st row of laticifers**

Clones	Number of laticifer rows in SB			Number of laticifer rows in IHB			Inter laticifer row distance in SB (mm)		Inter laticifer row distance in IHB (mm)		Distance from cambium to 1st LVR (mm)	
	Mean	CV (%)	%	Mean	CV (%)	%	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Gl 1	11.72	40	37.28	19.72	43	62.72	0.11	29	0.09	38	0.15	55
GT 1	8.83	62	27.26	23.56	43	72.74	0.12	42	0.09	15	0.19	48
PB 235	15.00	33	36.88	25.67	49	63.12	0.08	40	0.11	14	0.42	65
PB 28/59	20.06	44	51.94	18.56	57	48.06	0.07	11	0.09	27	0.21	39
PB 86	6.89	48	21.09	25.78	28	78.91	0.13	42	0.11	25	0.23	35
RRII 105	16.33	55	42.42	22.17	47	57.58	0.08	20	0.07	28	0.19	57
RRII 300	9.56	42	49.71	9.67	61	50.29	0.11	51	0.12	24	0.20	57
RRIM 600	11.28	31	42.12	15.50	33	57.88	0.10	34	0.13	32	0.18	45
RRIM 703	9.78	49	27.86	25.33	29	72.14	0.09	54	0.08	20	0.22	66
Tjir I	12.67	31	42.55	17.11	27	57.45	0.11	23	0.13	29	0.11	31
V R (F)	3.61**			2.69*			2.04 N.S		4.94**		1.36 N.S	
CD (5%)	6.12			9.51					0.03			

*Significant for $p < 0.05$; **Significant for $p < 0.01$; N.S: Not significant

IHB, the average distance between them did not show much variation.

Distance between cambium and first row of laticifers

Laticifers are differentiated from the fusiform initials of the cambium, in the form of concentric rings, alternating with other phloic elements such as sieve tubes, companion cells, phloem fibres, axial parenchyma and ray parenchyma. Due to the continued activity of the vascular cambium new laticifers are differentiated and the older ones are pushed outwards. The mean distance between cambium and the first row of laticifers is depicted in Table 1. The distance was the highest in PB 235 (0.42 mm) and the lowest in Tjir 1 (0.11 mm). Other clones had the mean distance ranging from 0.15 mm – 0.23 mm. Five clones *viz*., PB 235, RRIM 703, RRII 300, RRII 105 and Gl 1 depicted high CV values indicating high tree-to-tree variation. But rest of the clones had medium tree-to-tree variation. However, the variation between clones was not significant (Table 1).

Density of latex vessels contiguous to phloic rays

The number of latex vessels within a row in unit distance is termed as the density of latex vessels. Gomez *et al*. (1972) reported higher density in the soft bark than in the hard bark and this trait has been identified as a potential trait useful in crop improvement programs (Abraham *et al*., 1992). The density of latex vessels contiguous to phloic rays (Table 2) was maximum (25.44) in GT 1 (Fig. 1g) and minimum (22.73) in PB 235 (Fig. 1 h). In all the clones the tree-to-tree variation was very low. The clonal variation was statistically significant where the clones GT 1, Gl 1 and RRII 300 were superior to PB 86 and PB 235 (Table 2). Similarly the clones, Tjir 1, RRII 105, RRIM 600, PB 28/59 and RRIM 703 were also superior to PB 235. The

Table 2. **Density, diameter, laticifer area index and frequency of inter-connections between latex vessels**

Clones	LV density contiguous to rays row $^{-1}$mm^{-1}			LV density non contiguous to rays row $^{-1}$mm^{-1}			Total LV ensity row $^{-1}$mm^{-1}		Frequency of interconnections Unit area^{-1}		Diameter of LV (μm)		Laticifer area index	
	Mean	CV (%)	%	Mean	CV (%)	%	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)	Mean	CV (%)
Gl 1	25.27	4	88.11	2.85	23	10.55	28.12	4	18.54	7	25.52	12	43.15	45
GT 1	25.44	2	85.57	4.29	9	14.43	29.73	3	19.59	8	22.51	6	39.31	35
PB 235	22.73	3	86.10	3.96	9	14.14	26.69	3	17.64	11	24.72	15	76.17	58
PB 28/59	24.30	5	89.93	2.48	28	9.26	26.79	5	15.73	9	25.92	8	58.50	31
PB 86	23.72	4	88.87	4.28	6	14.92	28.00	4	18.71	13	24.99	7	46.18	33
RRII 105	24.65	4	88.32	2.37	27	8.88	27.02	4	22.11	6	23.64	7	37.91	45
RRII 300	24.95	4	88.73	2.96	29	10.65	27.91	6	20.94	15	25.49	13	27.62	50
RRIM 600	24.62	4	88.59	4.07	6	14.47	28.68	4	19.58	3	25.56	9	35.07	25
RRIM 703	24.13	8	90.07	2.27	35	8.60	26.40	7	18.20	20	21.63	7	29.80	24
Tjir I	24.78	2	88.50	3.01	14	10.78	27.79	2	19.16	4	24.06	15	39.09	44
V R (F)	4.42**			9.01**			5.39**		7.15**		3.19**		5.05**	
CD (5%)	1.12			0.80			1.31		1.95		2.38		19.14	

**Significant for p < 0.01

occurrence of more number of latex vessels contiguous to rays in this study categorically ascertains the close functional association of phloem rays and latex vessels.

Density of latex vessels non-contiguous to phloic rays

The density of latex vessels non-contiguous to phloic rays was considerably reduced in comparison to those latex vessels contiguous to rays in all the clones (Table 2). GT 1 (Fig. 2a) and PB 86 recorded high density (4.29 and 4.28) and the lowest density (2.27) was noticed in RRIM 703 (Fig. 2b). The tree-to-tree variation for this character was low in all the clones except in RRIM 703, which showed medium variation. However the clonal variability was statistically significant (Table 2). The clones GT 1, PB 86, RRIM 600 and PB 235 were statistically superior to Tjir 1, RRII 300, Gl 1, PB 28/59, RRII 105 and RRIM 703.

Total density of latex vessels

The total density of latex vessels per row per mm distance (Table 2) was the sum of the density of laticifers contiguous to rays and non-contiguous to rays. It was maximum (29.73) in GT 1 and minimum (26.40) in RRIM 703. Within clones the tree-to-tree variation was not significant. ANOVA for this character revealed significant clonal variability where GT 1, RRIM 600, Gl 1, PB 86, RRII 300 and Tjir 1 were statistically superior to RRIM 703 (Table 2). Gomez *et al.* (1972) reported significant clonal differences in the density of latex vessels within a row. It has been reported that the number of inter connections per unit length of latex vessels was independent to density as well as diameter (Premakumari *et al*., 1984). In the present study, latex vessels contiguous to rays and non-contiguous to rays have been treated separately for analysis and observed that 90 per cent of the laticifers are distributed in the vicinity of rays and the remaining 10 per cent situated away from the rays. The individual latex vessels within a row are interconnected to form articulated anastomosing structure around the phloic rays. Hence, it is reasonable to believe that the distribution pattern of laticifers is in tune with the inclination and orientation of phloic rays.

Frequency of inter-connections

Interconnections between latex vessels are formed by the dissolution of end walls of adjacent latex vessels and hence this character has been accounted as an interclonal variability trait (Premakumari *et al*., 1996). The frequency of interconnections may be increased due to the increase in the density of latex vessels. The articulated anastomosing nature of the laticiferous system in *Hevea*, is also correlated with tree girth (Premakumari *et al*., 1992). The frequency of interconnections (Table 2) between adjacent latex vessels within a row was maximum (22.11) in RRII 105 (Fig. 2c) and minimum (15.73) in PB 28/59 (Fig. 2d). RRII 300 (20.94) occupied the second position followed by GT 1 (19.59), RRIM 600 (19.58) and Tjir 1 (19.16). Tree-to-tree variation for the character was very low in all the clones. RRII 105 was statistically superior to eight clones *viz*., GT 1, RRIM 600, Tjir 1, PB 86, Gl 1, RRIM 703, PB 235 and PB 28/59 for this character (Table 2). Similarly RRII 300 exhibited superiority over PB 86, Gl 1, RRIM 703, PB 235 and PB 28/59; GT 1 was superior to PB 235, PB 28/59; and RRIM 600, Tjir 1, PB 86, Gl 1, RRIM 703 were superior over PB 28/59.

Diameter of latex vessels

Diameter of latex vessels has been considered as the most influential character on yield in *Hevea* clones (Frey-Wyssling, 1930; Sethuraj, 1977; Markose, 1984; Premakumari *et al.* 1992). Significant clonal variability for this trait has been reported earlier (Gomez *et al*., 1972; Gomez, 1982; Henon and Nicolas, 1989). The diameter of latex vessels (Table 2) was maximum (25.92 μm) in PB 28/59 (Fig. 2e) and minimum (21.63 μm) in RRIM 703 (Fig. 2f). The low CV values indicated low tree to tree variation for this trait. However, analysis of variance revealed significant clonal variability. Five clones *viz*., PB 28/59, RRIM 600, Gl 1, RRII 300, PB 86 were superior to GT 1 and RRIM 703 (Table 2). Clone PB 235 and Tjir 1 were statistically superior to RRIM 703.

Total cross sectional area of laticifers (Laticifer area index)

For the efficient exploitation of *Hevea* bark tissue for latex yield, the maximum number of latex vessels should be opened for a given length of tapping cut (Gomez *et al*., 1972). The total number of latex vessels at a given cross section of the bark may be taken as nfG, were 'n' is the number of latex vessel rings, 'f' is the mean density of latex vessels per mm distance within a ring and 'G' is the girth of the tree (Gomez, 1982). Hence the total cross sectional area of latex vessels would be nfG ($ðr^2$) where 'r' is the radius of latex vessels, which can be termed as laticifer area index.

The total cross sectional area of latex vessels was the highest in PB 235 (76.17) followed by PB 28/59 (58.50) and lowest in RRII 300 (27.62). Tree-to-tree variation was high in PB 235, whereas RRIM 600 and RRIM 703 exhibited low level of variation. However the remaining seven clones

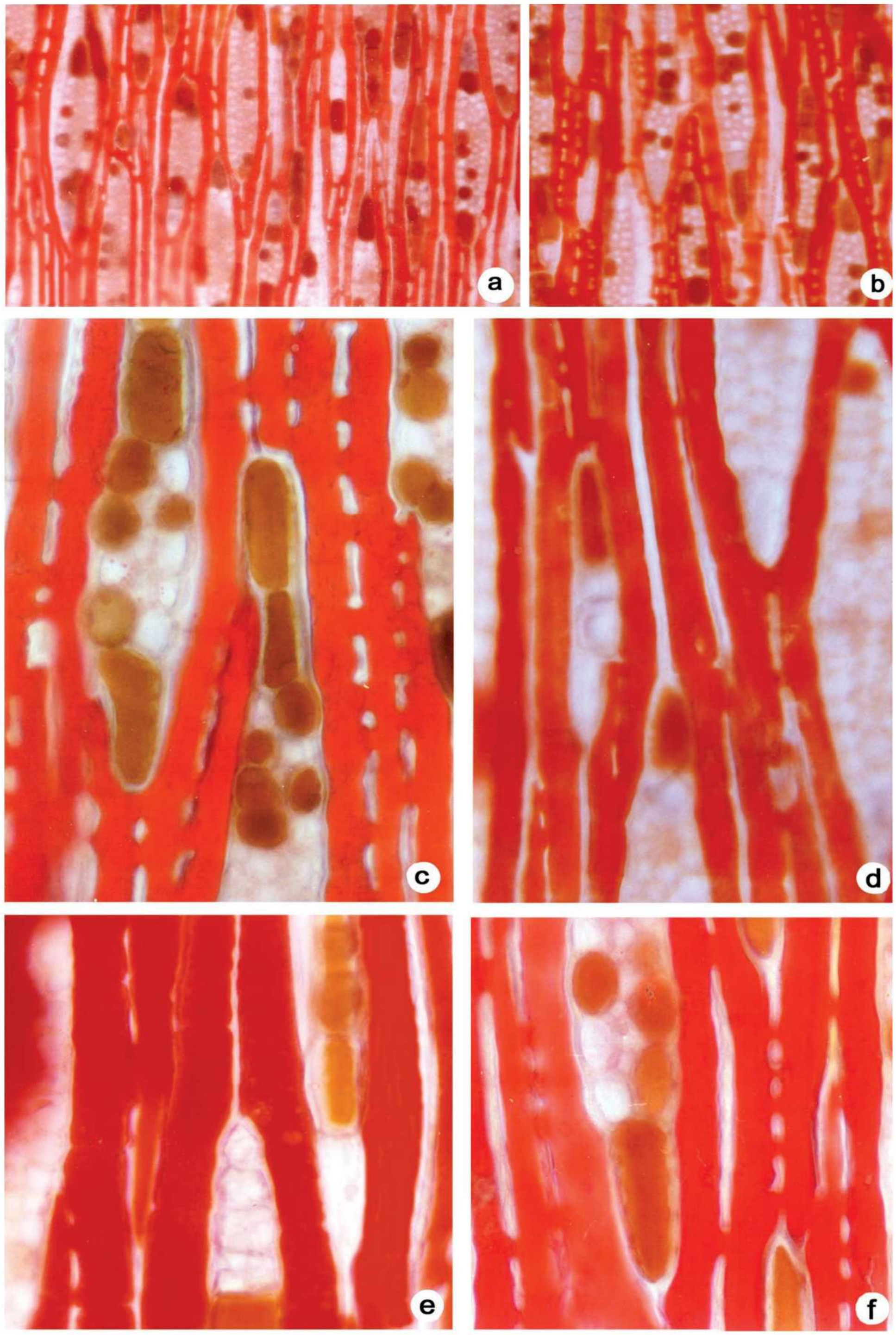

Fig. 2. a-f. Bark sections stained with Oil Red O. a- GT-1 maximum density of latex vessels non-contiguous to rays. b- RRIM 703 minimum density of latex vessels non-contiguous to rays. c- RRII 105 with maximum frequency of interconnections. d- PB 28/59 minimum frequency of interconnections. e- PB 28/59 maximum latex vessel diameter. f- RRIM 703 minimum latex vessel diameter. a&b- RLS, X75; c-d TLS, c&d- X200; e&f – X300

exhibited medium tree-to-tree variation. ANOVA for this character showed significant clonal variability where PB 235 and PB 28/59 were statistically superior to GT 1, Tjir 1, RRII 105, RRIM 600, RRIM 703 and RRII 300 (Table 2). Clonal variability in the laticifer area index as observed in the present study was concomitant with the earlier report of Premakumari *et al.* (1993). This character has also been related to the running direction of laticifers (Premakumari *et al*., 1988).

CONCLUSION

Laticifers, one of the important components of the bark of *H. brasiliensis* exhibited considerable clonal variation with respect to most of their characters investigated at present. Anatomical characters that exhibited clonal variation were number of laticifer rows and inter laticifer row distance in both soft bark and inner hard bark region, latex vessel density contiguous and non contiguous to rays, total latex vessel density, frequency of interconnection between latex vessels, diameter of latex vessels and laticifer area index. Two characters displayed non-significant variations were inter laticifer row distance and distance from cambium to the first laticifer row. The distribution of latex vessels in the soft bark region compared to the inner hard bark region was a noteworthy feature. This study also conclusively proved the close association of latex vessels with phloic rays in their distribution pattern and anatomically it was evident by the increased frequency of latex vessels contiguous to rays when compared to non-contiguous to rays.

ACKNOWLEDGEMENT

The first author is thankful to the University Grants Commission for providing financial assistance.

REFERENCES

Abraham, S.T. , Reghu, C.P., Madhavan, J., George, P.J., Potty, S.N., Panikkar, A.O.N. and Saraswathy, P. (1992). Evaluation of *Hevea* germplasm: 1. Variability in early growth phase. *Indian Jorunal of Natural Rubber Research,* **5**(1&2): 195-198.

Ashplant, H. (1928). Investigations into *Hevea* anatomy. *The Planters' Chronicle*, **23**: 469.

Bobilioff, W. (1920). Correlation between yield and number of latex vessel rows of *Hevea brasiliensis*. *Archief voor de Rubbercult'pur*, **4**: 391.

Bobilioff, W. (1923). Anatomy and Physiology of *Hevea brasiliensis*. *Zurich. Institute Orell Fussli.*

Bryce, G. and Campbell, L.E. (1917). On the mode of occurrence of latex vessels in *Hevea brasiliensis.* Department of Agriculture, Ceylon, *Bulletin*, No. **30.**

Bryce, G. and Gadd, C.H. (1923). Yield and growth in *Hevea brasiliensis*. Department of Agriculture. Ceylon, *Bulletin,* No. **68.**

Costa, R.B.D., Resende, M.D.V.D., Araujo, A.J.D., Goncalves, D.S.P. and Martins, A. L. M. (2000). Genotype-environment interaction and the number of test sites for the genetic improvement of rubber trees (*Hevea*) in Sao Paulo State, Brazil. *Genetics and Molecular Biology*, **23**(1): 179-187.

Frey-Wyssling, A. (1930). Investigation into the relation between the diameter of the latex tubes and the rubber production of *Hevea brasiliensis*. *Archief voor de Rubbercult'pur*, **14**: 135.

Gomez, J.B. (1966). *Electron microscopic studies on the development of latex vessels in* Hevea brasiliensis Mull. Arg. Ph. D. Thesis, University of Leeds.

Gomez, J.B. (1982). Anatomy of *Hevea* and its influence on latex production. Malaysian Rubber Research and Development Board, Kuala Lumpur. *Monograph No. 7*.

Gomez, J.B., Narayanan, R. and Chen, K.T. (1972).

Some structural factors affecting the productivity of *Hevea brasiliensis*: I. Quantitative determination of the laticiferous tissue. *Journal of Rubber Research Institute of Malaya*, **23**(3): 193-203.

Goncalves, P.D.S., Martins, A.L.M., Bortoletto, N. and Carvalho, A.Z. (1995). Broad sense heritability values and possible genetic gains in clonal selections of *Hevea*. *Brazilian Journal of Genetics*, **18**(4): 605-609.

Gottardi, M. V.C., Goncalves, P.D.S., Cardoso, M. and Mente, E.M. (1995). Genotypic and phenotypic correlations among characters of the mature rubber tree. *Cientifica (Jaboticabal)*, **23** (1): 53-64.

Henon, J.H. and Nicolas, D. (1989). Relation between anatomical organisation of the latex yield: Search for early selection criteria. *Physiology of rubber tree latex*, CRC Press Inc., Boca Raton, Florida, pp. 31-50.

Ho, C.Y. (1972). *Investigations on shortening the generative cycle for yield improvement in* Hevea brasiliensis. M.Sc. Thesis, Cornel University, U.S.A.

Ho, C.Y. (1976). Clonal characters determining the yield of *Hevea brasiliensis*. *Proceedings of the International Rubber Conference,* 1975, Kuala Lumpur, Malaysia, **2**: 27-38.

Ho, C.Y., Narayanan, R. and Chen, K.T. (1973). Clonal nursery studies in *Hevea* I. Nursery yields and associated structural characteristics and their variations. *Journal of Rubber Research Institute Malaysia*, **23**(4): 274.

Markose, V.C. (1984). *Biometrical analysis of yield and certain yield attributes in the para rubber tree* Hevea brasiliensis Muell. Arg. Ph.D. Thesis, Kerala Agricultural University, Trivandrum, India, 119 p.

Narayanan, R., Gomez, J.B. and Chen, K.T. (1973). Some structural factors affecting productivity of *Hevea brasiliensis* II. Correlation studies between structural factors and yield. *Journal of Rubber Research Institute of Malaysia,* **23**: 285-297.

Narayanan, R., Ho, C.Y. and Chen, K.T. (1974). Clonal nursery studies in *Hevea* III. Correlation between yield, structural characters, latex constituents and plugging index. *Journal of Rubber Research Institute of Malaysia,* **24**(1): 1-14.

Omman, P. and Reghu, C.P. (2003). Staining procedure for laticiferous system of *Hevea brasiliensis* using Oil Red O. *Indian Journal of Natural Rubber Research*, **16**(1&2): 41-44.

Omman, P. and Reghu, C.P. (2008). Inclination of laticifers and phloic rays in ten clones of Hevea brasiliensis. *Indian Journal of Natural Rubber Research*, **21**(1&2): 47-66.

Paiva, J.R.D., Rossetti, A.G. and Goncalves, P.D.S. (1982). Use of path coefficient in *Hevea brasiliensis* breeding. *Pesquisa Agropecuaria Brasileira*, **17**(3): 433-440.

Premakumari, D., Marattukalam, J.G. and Panikkar, A.O.N. (1988). Influence of the orientation of laticiferous tissue on yield in *Hevea brasiliensis Muell*. Journal of Plantation Crops, **16**(1): 12-18.

Premakumari, D. and Panikkar, A.O.N. (1989). An attempt to improve test tapping in *Hevea* seedlings. *Journal of Plantation Crops*, **18** (Supplement): 383-387.

Premakumari, D., Panikkar, A.O.N. and George, P.J. (1992). Comparative anatomy of virgin and renewed bark in *Hevea*. *Journal of Plantation Crops,* **20** (Supplement): 204-206.

Premakumari, D., Panikkar, A.O.N., Marattukalam, J.G. and Sethuraj, M.R. (1993). Interclonal variability, correlations and genetic parameters of certain anatomical and physiological characters and their importance as selection criteria for drought tolerance in *Hevea brasiliensis*. *Plant Physiology and Biochemistry*, **20**(2): 122-126.

Premakumari, D., Panikkar, A.O.N., Marattukalam, J.G. and Sethuraj, M.R. (1996). Yield and anatomical characters in *Hevea*: A path coefficient analysis and characterization of clones. *Indian Journal of Natural Rubber Research*, **9**(1): 12-16.

Premakumari, D., Panikkar, A.O.N. and Shankar, S. (1991). Comparative bark anatomy of three induced polyploids of *Hevea brasiliensis* a (willd. ex.a.juss). *Journal of Plantation Crops,* **18** (supplement): 17-23.

Premakumari, D., Sasikumar, B., Panikkar, A.O.N. and Marattukalam, J.G. (1984). Variability and association of certain bark anatomical traits in *Hevea brasiliensis* Muell Arg. *PLACROSYM VI*: 49-54.

Sanderson, A.R. and Sutcliffe, H. (1929). Vegetative characters and yield of *Hevea*. *Journal of Rubber Research Institute of Malaysia,* **1**: 75.

Sethuraj, M.R. (1977). *Studies on the physiological factors influencing yield in* Hevea brasiliensis Muell. Arg. Ph.D. Thesis, Banarus Hindu University, U.P. India, 184 p.

Sethuraj, M.R. (1981). Yield components in *Hevea brasiliensis*: Theoretical considerations. *Plant, cell and environment*, **4**: 81-83.

Southorn, W.A. (1966). Electron microscopic studies on the latex of *Hevea brasiliensis*. *Proceedings of the 6th International Congress on Electron Microscopy,* Kyoto. **2**: 385 p.

Vischer,W. (1921). Over de resultaten verkregen met het oculeeren van *Hevea brasiliensis* op de onderneming pasir waringen. (Results obtained with budded trees of *Hevea brasiliensis* on Pasir Waringen estate). *Archief voor de Rubbercult'pur*, **5**: 17 p.