



Correlation of anatomical characters in the bark of *Hevea brasiliensis*

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Abstract

Hevea brasiliensis is an important tree that serves as the Natural Rubber source for commercial utilisation. The exploitation of latex from *Hevea* is by controlled wounding of the bark of the plant called tapping. The bark tissue system, which is formed as secondary phloem during the secondary growth of the plant, is the central tissue system that contains latex-containing latex vessels. Therefore, anatomically the bark characters have a pivotal physiological role in the latex production in the latex vessels and thereby its commercial exploitation. The present investigation aims to understand the interrelationship of various anatomical factors in nine prominent clones of *Hevea*. Statistical analysis proved that characters including laticifer area index, the girth of the plant, total bark thickness, inner hard bark thickness, stone cell area in inner hard bark, sieve tube diameter, total latex vessels density, number of stone cell rows in inner hard bark have significant interrelation. The most significant outcome of this study is that these statistical inferences can be used to find out the selection of clones in *Hevea*.

Keywords: *Hevea brasiliensis*, bark anatomy, secondary phloem, latex vessels, correlation

Introduction

Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell. Arg. represents the family Euphorbiaceae, the major contributor of Natural Rubber (NR). The specialized tissue system known as latex vessels or laticiferous system present in the secondary phloem or bark tissue serves as site and carrier cells to latex in *Hevea*. For commercial exploitation of latex from *Hevea*, latex vessels were cut by a process called tapping, which is performed by a standard half spiral cut from the upper left to the lower right of the tree. In *Hevea*, the secondary phloem, which is also named the bark, consists of three regions, the innermost region close to the cambium, the soft bark (SB), inner hard bark (IHB) and outermost outer hard bark (OHB), functioned as the part of the protective region of the stem (Omman and Reghu, 2013b).

Anatomically many factors are related independently and function in relation to each in the bark *Hevea* tree. Tree girth has been identified as one of the most important characters of latex yield in *H. brasiliensis* (Ho *et al.*, 1973; Narayanan *et al.*, 1973; Premakumari *et al.*, 1997) [23, 24, 34]. Premakumari *et al.*, (1997) [34] reported that girth increment on tapping was negatively correlated with the density of laticifers and phloic ray characteristics. Narayanan *et al.* (1974) [25] investigated the interrelationships of various structural characters and observed a linear correlation of girth with bark thickness, number of laticifer rows and yield.

Total bark thickness comprises the thickness of whole bark tissue that externally surrounds the *Hevea*'s wood. It has been identified as clonal characteristics and was related to laticifer rows. (Gomez and Chen, 1967; Gomez *et al.*, 1972; Narayanan *et al.*, 1974) [25]. The thickness of bark also influenced the yield of *Hevea* clones (Narayanan *et al.*, 1973 [23, 24]; Ho *et al.*, 1973; Gottardi, 1995 and Goncalves *et al.*, 2004) [25]. The laticifers are found in the secondary phloem in rows. The laticifer rows have been reported as a

quantitative anatomical parameter of latex yield in *H. brasiliensis* (Bobilihoff, 1923; Gomez, 1966). Many workers have proved the correlation of this trait with yield in *Hevea* (Bobilihoff 1920; La Rue, 1921; Taylor, 1926; Narayanan *et al.*, 1973; Narayanan *et al.*, 1974) [8, 21, 43, 22].

The distance between laticifer rows has been considered a yield determining character in *Hevea* (Paiva *et al.*, 1982) [29]. Gomez *et al.* (1972) noted considerable variation in the average distance between laticifer rows in different clones. Narayanan *et al.* (1974) [25] observed a positive correlation between girth and the average distance between laticifer rows. Gottardi (1995) [15] noted significant genotypic and phenotypic correlations among different bark characters, including the distance between consecutive rows of laticifers. Narayanan *et al.* (1974) [25] observed a positive correlation between girth and the average distance between laticifer rows. Gottardi (1995) [15] noted significant genotypic and phenotypic correlations among different bark characters, including the distance between consecutive rows of laticifers. Reghu *et al.* (1996) reported a wide range of variability in many structural characters of the bark, including the density of latex vessels in wild *Hevea* germplasm. The significant genotypic and phenotypic variation in the density of latex vessels has also been reported (Gottardi, 1995) [15]. The relationship between the density of latex vessels and the width of phloic rays has also been reported earlier (Premakumari *et al.*, 1984).

Clonal variability in the frequency of interconnections between latex vessels has been reported by Premakumari *et al.* (1984; 1991) and opined that this trait had only low or moderate genetic advance along with high heritability estimates. The authors further pointed out that the number of interconnections per unit distance within the laticifer rows depends on the density and diameter of latex vessels. Latex vessel diameter has been reported as an important factor determining the latex yield (Ashplant, 1927; 1928a; 1928b; 1928c). Simple correlations among yield, girth, bark

thickness, number of laticifer rows and diameter of latex vessels have been reported earlier by various researchers. (Gomez *et al.*, 1972; Ho *et al.*, 1973; Narayanan *et al.*, 1973; Ho, 1972, 1976; Sethuraj *et al.*, 1981; Premakumari and Panikkar, 1989).

Premakumari *et al.* (1984) reported a negative correlation of ray width with latex vessel density. A significant increase in ray height in drought-tolerant trees has been reported by Premakumari *et al.* (1993 a). Anisio *et al.* (1998) studied the diameter of sieve tubes in 15 *Hevea* clones and reported a significant correlation with rubber production. The relationship between the diameter of sieve tubes and yield has also been well established (Fernado and Tambiah, 1970; Gunnery, 1935). The various anatomical characters associated with the secondary phloem in *Hevea* were published earlier by Omman and Reghu (2013a and 2013b). A survey on the anatomical investigation done in *Hevea* with statistical analysis revealed a strong correlation between several anatomical characters. Hence the present investigation and analysis were undertaken to understand the relationship between nine prominent *Hevea* clones for twenty-one characters.

Materials and Methods

Nine Wickham clones of *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. were selected from the Germplasm gardens of Rubber Research Institute of India, Chetthelal, Ranni, Kerala. The experimental station is at 9° 22' N latitude and 76° 50' E longitude with an altitude of 80m above the MSL. These germplasm gardens are planted in Randomised Block Design (RBD) with three replicates and three trees per plot. The trees were under regular tapping and had 17-21 years. Nine mature trees from each clone (three trees per replication) were selected to study the structure of the bark.

For the present study virgin bark samples were collected from the selected trees at 150 cm height (for mature trees) from the ground. The samples collected were fixed in formalin-acetic-alcohol (FAA) and were sectioned at 30 – 60 µm thickness at different planes *viz.* cross-sectional (CS), tangential longitudinal (TLS) and radial longitudinal (RLS) plane, using Reichert Jung sledge microtome. Sections were stained with Oil Red O (Omman and Reghu, 2003) and mounted in 50% glycerin. The bark sections were observed under the Leitz Aristoplan Research microscope attached to Leica Q 5000 I W Image Analysis System.

The images of the bark sections documented in the Image Analysis System were used to measure the inclination of laticifers / phloic rays and other anatomical traits using Leica Q Win V.2.1 Image analysis software. The TLS of the bark was used to measure the density and diameter of laticifers and the frequency of interconnections. Cross-section and RLS were used to count the number of laticifer rows, inter-row distances, area occupied by stone cells, and soft/hard bark thickness. Observations from ten microscopic fields were taken per plant for each anatomical parameter. The simple correlation was worked out to find out the relationship among themselves and between different characters. For statistical analysis of data, software of excel (MS office) and SPSS 10 were used.

The various anatomical characters studied and subjected to statistical correlation analysis include, Sieve tube length (STL), Sieve tube diameter (STD), Latex vessel diameter (LV Dia), Frequency of interconnections /unit area (FIC),

Total vessel density (TLV Den), Latex vessel density contiguous to rays (LVD CR), Latex density non-contiguous to rays (LVD NCR), Distance from cambium to 1st latex vessel row (DC 1LVR), Soft bark thickness (SBT), number of latex vessel rows in soft bark (NLVR SB), distance between adjacent rows in SB (DR SB), IHB- Thickness of inner hard bark (IHB), Number of latex vessel rows in inner hard bark (NLVR IHB), Distance between adjacent rows in inner hard bark (DR IHB), Number of stone cell rows in inner hard bark NSR IHB), Thickness of Outer hard bark (OHBT), TBT- Total bark thickness (TBT), Stone cell area in inner hard bark (SCA IHB), Stone cell area in Hard bark (SCA HB), Girth of the tree Girth), and total cross sectional area of latex vessels (LAI- Laticifer Area Index).

Result and Discussion

The correlation of various characters is represented in Table 1. For example, sieve tube length was positively correlated with sieve tube diameter (0.504) and stone cell area in HB (0.373) and significant negative association with LVs density contiguous to rays (-0.340) and outer hard bark thickness (-0.426). However, sieve tube diameter showed only a positive significant association with LVs density non-contiguous to rays (0.404) and IHB thickness (0.328). The literature survey indicates no more such observation earlier in *Hevea*.

LV diameter had a significant positive influence on the thickness of SB (0.326) and the total cross-sectional area of latex vessels (0.449). Latex vessel diameter has been reported as an important factor determining the latex yield (Ashplant, 1927; 1928a; 1928b; 1928c). Simple correlations among yield, girth, bark thickness, number of laticifer rows and diameter of latex vessels have been reported earlier by various researchers. (Gomez *et al.*, 1972; Ho *et al.*, 1973; Narayanan *et al.*, 1973; Ho, 1972, 1976; Sethuraj *et al.*, 1981; Premakumari and Panikkar, 1989) [22, 30]. Moreover, the radius of latex vessels has been used as an important variable to ascertain the laticifer area index, the potential quantitative anatomical parameter used for breeding and selection programmes (Gomez *et al.*, 1972).

The frequency of interconnections exhibited significant positive correlations with total LVs density (0.311) and LV density contiguous to rays (0.313). Also, it had negative correlations with total bark thickness (0.330), girth (0.407) and total cross-sectional area of LVs (0.459). Total LVs density possessed very high significant positive correlations with LVs density contiguous to rays (0.784), LVs density non-contiguous to rays (0.556) and the area occupied by stone cells in outer HB (0.474). However, the density of laticifers non-contiguous to rays was positively correlated with stone cell area in IHB (0.447) and stone cell area in outer HB (0.664). The number of latex vessels within a row in the unit distance is termed the density of latex vessels. Gomez *et al.* (1972) reported higher density in the soft bark than that of hard bark, and this trait has been identified as a potential trait for crop improvement programs (Abraham *et al.*, 1992). The relationship between the density of latex vessels and the width of phloic rays has also been reported earlier (Premakumari *et al.*, 1984) [35].

The thickness of the SB had significant positive correlations with the number of LV rows in SB (0.848) and the total cross-sectional area of LVs (0.425). The thickness of IHB recorded a highly significant positive correlation with many characters, *viz.* number of LVs rows in IHB (0.879), number

of stone cell rows in IHB (0.633), total bark thickness (0.433), girth (0.491) and total cross-sectional area of LVs (0.554). Outer hard bark thickness showed a very high positive association with total bark thickness (0.859) and negative association with stone cell area in IHB (-0.366).

Total bark thickness also made a similar significant negative correlation with stone cell area in IHB (-0.308) and very high positive correlation with tree girth (0.560) and total cross-sectional area of LVs (0.507). The outer hard bark thickness has a positive correlation with total bark thickness (0.859) but negatively correlated with sieve tube length (0.426), stone cell area in inner hard bark (0.366). Various workers have reported the relationship between traits like bark thickness and laticifer rows (Bobilioff, 1923; Gomez *et al.*, 1972; Narayanan *et al.*, 1973). Studies carried out by Narayanan *et al.* (1974) proved that bark thickness has been related to girth, the number of latex vessel rows and distance between laticifer rows.

The number of LV rows in SB showed a significant negative association with many characters *viz.* distance between LVs rows in SB (-0.476), IHB thickness (-0.332), stone cell area in HB (-0.338) and positively with the total cross-sectional area of LVs (0.381). Similarly, such type of significant positive correlations was noted between the number of LV rows in IHB with the inner hard bark thickness (0.879), the distance between rows in inner hard bark (0.523), number of stone cell rows in inner hard bark (0.550), total bark thickness (0.518), the girth of the plant (0.447) and laticifer area index (0.584). Premakumari *et al.* (1993a) recorded a significant reduction in the number of laticifer rows in the soft bark compared to the hard bark. Due to the high correlation of yield with the number of laticifer rows, considerable emphasis has been given to selecting high yielding clones based on the number of laticifer rows in *Hevea* (Rubber Research Institute Malaya, 1966; Wycherly, 1969). The number of laticifer rows has been reported as a quantitative anatomical parameter of latex yield in *H. brasiliensis* (Bobilioff, 1923; Gomez, 1966)^[8]. Many workers have proved the correlation of this trait with yield in *Hevea* (Bobilioff 1920; La Rue, 1921; Taylor, 1926; Rubber research institute, Malaya, 1963, 1964, 1966, 1968; Narayanan *et al.*, 1973; Narayanan *et al.*, 1974)^[7, 21, 43, 22]. Hamzah *et al.* (1975)^[17] reported a negative correlation between the number of laticifer rows and inter-row distance. The distance between LV rows in IHB had a significant positive correlation with stone cell area in IHB (0.501) and stone cell area in outer hard bark (0.509) and also had a significant negative association with total bark thickness (0.363) and total cross-sectional area of LVs (-0.321). Narayanan *et al.* (1974)^[25] observed a positive correlation between girth and the average distance between laticifer rows. Gottardi (1995)^[15] noted significant genotypic and phenotypic correlations among different bark characters, including the distance between consecutive rows of laticifers. A positive correlation between the number of laticifer rows and the initial flow rate was reported earlier (Sethuraj *et al.*, 1974). The number of stone cell rows in IHB recorded very high positive and significant associations with total bark thickness (0.308), the girth of the tree (0.358) and total cross-sectional area of LVs (0.359). Stone cell area in outer hard bark has noted a significant correlation with sieve tube length (0.373), total latex vessel density (0.474), latex vessel density non-contiguous to rays (0.664), the distance between latex vessel rows in inner hard bark

(0.509), stone cell area in inner hard bark (0.611) but negatively correlated with the number of latex vessel rows soft bark (0.338). The girth of the plant recorded a positive correlation with many of the anatomical parameters, including inner hard bark thickness (0.491), number of latex vessels rows in inner hard bark (0.447), number of stone cell rows inner hard bark (0.358), total bark thickness (0.560), laticifer area index (0.759) but it has a negative correlation with only one factor, the frequency of interconnection of latex vessels (0.407). Premakumari *et al.*, (1997) reported that girth increment on tapping was negatively correlated with the density of laticifers and phloic ray characteristics. Narayanan *et al.* (1974)^[25] investigated the interrelationships of various structural characters and observed a linear correlation of girth with bark thickness, number of laticifer rows and yield. The relationship of yield and girth has been confirmed in mature trees (Narayanan and Ho, 1970) and nursery clones (Narayanan and Ho, 1973). Hence girth has been considered a stable character for the location-specific selection of *Hevea* clones in different environments (Goncalves, 2004). According to Goncalves *et al.* (1989), girth did not correlate with plugging index but positively correlated with yield and bark thickness. Gomez *et al.* (1972)^[12] used girth as a vital variable to work out the laticifer area index, the most critical parameter to assess tapping efficiency. The laticifer area index was correlated positively with latex vessel diameter (0.449), soft bark thickness (0.425), number of latex vessel rows in soft bark (0.381), inner hard bark thickness (0.554), number of latex vessel rows in inner hard bark (0.584), number of stone cell rows in inner hard bark (0.359), total bark thickness (0.507) and girth (0.759). Significantly it was also correlated negatively with the frequency of interconnections (0.459) and distance between rows in inner hard bark (0.321). The Laticifer area index has been used as an essential parameter to determine the total cross-sectional area of laticifers cut open during tapping. Premakumari *et al.* (1993b) noticed significant clonal variability in the laticifer area index in *Hevea* clones. Reghu *et al.* (1996) recorded a higher laticifer area index in wild *Hevea* germplasm than RRII 105 and GT 1. Premakumari *et al.* (1993a) also reported the positive relationship of the laticifer area index with yield.

Conclusions

The present correlation study proved that many anatomical characters are interrelated. The anatomical characters having significant correlation include laticifer area index, the girth of the plant, total bark thickness, inner hard bark thickness, stone cell area in inner hard bark, sieve tube diameter, total latex vessels density, number of stone cell rows in inner hard bark. Other characters also indicated their significance and relationship both positively and negatively. The present investigation's findings will help scientists find out the role anatomical characters have value in the plant's yield. More precisely, these anatomical results will help researchers select high yielding clones in *Hevea*.

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Table 1: Correlation among various characters in Hevea

	STL	Std	LV dia.	FIC	TLV Den	LVD CR	LVD N CR	DC 1 LVR	SBT	NLVR SB	DR SB	IHBT	NLVR IHB	DR IHB	NSR IHB	OHB T	TBT	SCA IHB	SCA OHB	Girth	LAI
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
STL	1.000	0.504**	0.209	0.074	-0.131	0.340**	0.245*	0.198	0.063	0.023	-0.083	0.190	0.005	0.207	0.260*	0.426*	-0.263	0.274*	0.373*	0.212	0.274*
STD		1.000	0.207	0.014	0.125	-0.152	0.404**	0.033	-0.068	-0.114	0.074	0.328*	0.146	0.107	0.247*	-0.211	-0.043	0.091	0.247*	0.106	0.207
LV dia.			1.000	-0.200	-0.036	-0.098	0.073	0.050	0.326**	0.172	0.053	-0.039	-0.094	0.040	-0.107	0.011	0.086	0.058	0.167	0.157	0.449*
FIC				1.000	0.311**	0.313**	0.081	-0.073	0.233*	-0.213	0.075	-0.180	0.239*	0.191	-0.142	-0.194	0.330*	0.132	0.246*	0.407*	0.459*
TLV Den					1.000	0.784**	0.556**	0.302*	-0.168	-0.164	0.089	-0.068	-0.128	0.163	0.062	-0.098	-0.170	0.236*	0.474*	-0.140	-0.030
LVD CR						1.000	-0.080	0.292*	-0.098	-0.027	-0.026	0.265*	-0.173	-0.009	-0.107	0.053	-0.114	-0.051	0.073	0.272*	0.238*
LVD NCR							1.000	-0.094	-0.137	-0.227	0.177	0.246*	0.026	0.273*	0.242*	-0.229	-0.120	0.447*	0.664*	0.140	0.110
DC 1 LVR								1.000	0.162	0.031	-0.068	0.176	0.063	0.050	-0.106	-0.226	-0.066	0.112	-0.045	0.166	0.088
SBT									1.000	0.848*	-0.128	0.244*	-0.218	-0.094	-0.140	-0.098	0.085	0.007	-0.211	0.175	0.425*
NLVR SB										1.000	0.476*	0.332*	0.232*	-0.174	-0.218	-0.023	0.063	-0.120	0.338*	0.103	0.381*
DR SB											1.000	0.063	-0.102	0.292*	0.022	0.082	0.067	0.231	0.287*	0.034	-0.214
IHBT												1.000	0.879*	-0.174	0.633*	0.006	0.433*	0.027	-0.014	0.491*	0.554*
NLVR IHB													1.000	0.523*	0.550*	0.162	0.518*	-0.166	-0.228	0.447*	0.584*
DR IHB														1.000	-0.009	0.281*	0.363*	0.501*	0.509*	-0.100	0.321*
NSR IHB															1.000	0.038	0.308*	0.090	0.080	0.358*	0.359*
OHB T																1.000	0.859*	0.366*	0.241*	0.298*	0.120
TBT																	1.000	0.308*	0.281*	0.560*	0.507*
SCA IHB																		1.000	0.611*	0.019	-0.044
SCA OHB																			1.000	0.072	-0.074
Girth																				1.000	0.759*
LAI																					1.000

* Significant for $p < 0.05$ ** Significant for $p < 0.01$ 1. STL -Sieve tube length; 2.STD-Sieve tube diameter; 3.LV Dia- Latex vessel diameter; 4. FIC- Frequency of interconnections /unit area; 5. TLV Den -Total vessel density; 6. LVD CR -Latex vessel density contiguous to rays; 7. LVD NCR- Latex density noncontiguous to rays; 8. DC 1LVR -Distance from cambium to 1st latex vessel row, 9. SBT- Soft bark thickness; 10. NLVR SB- Number of latex vessel rows in soft bark; 11. DR SB- Distance between adjacent rows in SB; 12. IHB- Thickness of inner hard bark; 13. NLVR IHB- Number of latex vessel rows in inner hard bark; 14. DR IHB-Distance between adjacent rows in inner hard bark; 15. NSR IHB- Number of stone cell rows in inner hard bark; 16. OHBT- Outer hard bark thickness; 17. TBT- Total bark thickness; 18. SCA IHB- Stone cell area in inner hard bark; 19. SCA OHB- Stone cell area in Outer Hard bark; 20. Girth- Girth of the tree;21. LAI- Total cross sectional area of latex vessels (Laticifer Area Index).

References

1. Abraham ST, Reghu CP, Madhavan J, George PJ, Potty SN, Panikkar AON, et al. Evaluation of *Hevea* germplasm: 1. Variability in early growth phase. *Indian Journal of Natural Rubber Research*,1992;5(1-2):195-198.
2. Anisio A, Goncalves PDS, Tomas MAT. Sieve tube diameter and the rubber production in rubber tree clones. *Bragantia*,1998;57(1):57-60.
3. Ashplant H. Investigations into *Hevea* anatomy. *Bull. Rubb. Grow. Assn.*,1927;9:571.
4. Ashplant H. Investigations into *Hevea* anatomy. *The Planters' Chronicle*,1928;23:469.
5. Ashplant H. Latex tube bore. *Bull. Rubb. Grow. Assn.*1928;10:769.
6. Ashplant H. Yield variability in *Hevea brasiliensis*. *Nature*,1928;121:1018.
7. Bobilioff W. Correlation between yield and number of latex vessel rows of *Hevea brasiliensis*. *ArchRubbercult. Ned-Indie*,1920;4:391.
8. Bobilioff W. Anatomy and Physiology of *Hevea brasiliensis*. *Zurich. Institute OrellFussli*, 1923.

9. Fernando DM, Tambiah MS. Sieve tube diameters and yields in *Hevea* –D-spp. A preliminary study. *Quarterly Journal Rubber Research Institute, Ceylon*,1970:46(3-4): 88-92.
10. Gomez JB. Electron microscopic studies on the development of latex vessels in *Hevea brasiliensis* Mull. Arg. Ph. D. thesis, University of Leeds, 1966.
11. Gomez JB, Chen KT. Alignment of anatomical elements in the stem of *Hevea brasiliensis*. *Journal of Rubber Research Institute of Malaya*,1967:20(2):91-99.
12. Gomez JB, Narayanan R, Chen KT. Some structural factors affecting the productivity of *Hevea brasiliensis*: I. Quantitative determination of the laticiferous tissue. *Journal of Rubber Research Institute of Malaya*,1972:23(3):193-203.
13. Goncalves PDS, Cardoso M, Martins ALM, Lavorentic C. Correlations studies between plugging index yield, girth and bark thickness in *Hevea* clones. *Revista Brasileira de Genetica*,1989:12(3):589-604.
14. Goncalves PDS, Martins ALM, Bortoletto N, Saes LA. (2004). Selection and genetic gains for juvenile traits in progenies of *Hevea* in Sao Paulo State, Brazil. *Genetics and Molecular Biology*,2004:27(2):207-214.
15. Gottardi MVC, Goncalves PDS, Cardoso M, Mente EM. Genotypic and phenotypic correlations among characters of the mature rubber tree. *Cientifica (Jaboticabal)*,1995:23 (1): 53-64.
16. Gunnery H. Yield prediction in *Hevea*: A study of sieve tube structure in relation to latex yield. *Journal of Rubber Research Institute Malaya*,1935:6:8.
17. Hamzah S, Mahmood AA, Sivanadyan K, Gomez JB. Effects of mineral deficiencies on bark anatomy of *Hevea brasiliensis*. Proceedings of the International Rubber Conference, 1975, Kuala Lumpur, 1975, 165-180.
18. Ho CY. Investigations on shortening the generative cycle for yield improvement in *Hevea brasiliensis*. M.Sc. Thesis, Cornell University, U.S.A, 1972.
19. Ho CY. Clonal characters determining the yield of *Hevea brasiliensis*. *Proceedings of the International Rubber Conference*, 1975, Kuala Lumpur, Malaysia,1976:2:27-38.
20. Ho CY, Narayanan R, Chen KT. Clonal nursery studies in *Hevea* I. Nursery yields and associated structural characteristics and their variations. *Journal of Rubber Research Institute Malaya*,1973:23(4):274.
21. La Rue CD. Correlation in structure between mother and daughter trees of *Hevea*. *Archiefv. d. Rubbercult*,1921:5:567
22. Narayanan R, Gomez JB, Chen KT. Some structural factors affecting productivity of *Hevea brasiliensis* II. Correlation studies between structural factors and yield. *Journal of Rubber Research Institute of Malaya*,1973:23:285-297.
23. Narayanan R, Ho CY. Yield –girth relationship studies on *Hevea*. *Journal of Rubber Research Institute of Malaya*,1970:23(1):23.
24. Narayanan R, Ho CY. Clonal nursery studies in *Hevea* II. Relationship between yield and girth. *Journal of Rubber Research Institute of Malaya*,1973:23(5):332-338.
25. Narayanan R, Ho CY, Chen KT. Clonal nursery studies in *Hevea* III. Correlation between yield, structural characters, latex constituents and plugging index. *Journal of Rubber Research Institute of Malaysia*,1974:24(1):1-14.
26. Omman P, Reghu CP. Staining procedure for laticiferous system of *Hevea brasiliensis* using Oil Red O. *Indian Journal of Natural Rubber Research*,2003:16(1-2):41-44.
27. Omman P, Reghu CP. Clonal variability in the distribution of sieve tubes and companion cells in *Hevea brasiliensis* bark tissue. *Rubber Science*,2013:26(1):95-100.
28. Omman P, Reghu CP. Clonal variation in the quantitative traits of laticifers in *Hevea brasiliensis*. *Rubber Science*,2013:26(2):279-289.
29. Paiva JRD, Rossetti AG, Goncalves PDS. Use of path coefficient in *Hevea brasiliensis* breeding. *Pesquisa Agropecuaria Brasileira*,1982:17(3):433-440.
30. Premakumari D, Panikka AON. An attempt to improve test tapping in *Hevea* seedlings. *Journal of Plantation Crops*,1989:18:383-387.
31. Premakumari D, Panikkar AON, Marattukalam JG, Sethuraj MR. Comparative bark anatomy of drought tolerant and susceptible clones of *Hevea brasiliensis*. *Indian Journal of Natural Rubber Research*,1993:6(1-2):10-14.
32. Premakumari D, Panikkar AON, Marattukalam JG, Sethuraj MR. 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*,1963:20(2):122-126.
33. Premakumari D, Panikkar AON, Sethuraj MR, Marattukalam JG. Growth, yield and flow characters and their correlations with brown bast incidence in ten *Hevea* clones. *Indian Journal of Natural Rubber Research*,1991:4(2):107-113.
34. Premakumari D, Panikkar AON, Sethura MR, Marattukalam JG. Associations of structural traits: Yield, girth and occurrence of tapping panel dryness in *Hevea brasiliensis*. *Indian Journal of Natural Rubber Research*,1997:10(1-2):27-33.
35. Premakumari D, Sasikumar B, Panikkar AON, Marattukalam JG. Variability and association of certain bark anatomical traits in *Hevea brasiliensis* Muell Arg. *Placrosym*,1984:6:49-54.
36. Reghu CP, Abraham ST, Madhavan J, George PJ, Potty SN, Leelamma KP. Evaluation of *Hevea* germplasm: Variation in bark structure of wild Brazilian germplasm. *Indian Journal of Natural Rubber Research*,1996:9(1):28-31.
37. Rubber Research institute of Malaya. *Rep. Rubb. Res. Inst. Malaya*, 1962, 35.
38. Rubber Research institute of Malaya. *Rep. Rubb. Res. Inst. Malaya*, 1963, 30.
39. Rubber Research institute of Malaya. *Rep. Rubb. Res. Inst. Malaya*, 1965, 25.
40. Rubber Research institute of Malaya. *Rep. Rubb. Res. Inst. Malaya*, 1967, 26.
41. Sethuraj MR. Yield components in *Hevea brasiliensis*: Theoretical considerations. *Plant, cell and environment*,1981:4:81-83.
42. Sethuraj MR, Sulochamma S, George MJ. Influence of initial flow rate, rows of latex vessels and plugging index on the yield of the progenies of *Hevea*

- brasiliensis* Muell. Arg. derived from crosses involving Tjir 1 as the female parent. *Indian Journal of Agricultural Science*,1974:44:354-356.
43. Taylor RA. The interrelationship of yield and the various vegetative characters in *Hevea brasiliensis*. *Rubb. Res. Sch. Ceylon Bull.*, 43.
 44. Wycherley PR. Breeding of *Hevea*. *J. Rubb. Res. Inst. Malaya*,1969:21:38.
 45. Emuedo OA, Omokhafa KO, Uzunigbe EO, Ohikhena FU, Uwumarongie DA, Ubani SE, Chukwuka AN, Ugiagbe-Ekue U, Omorogbe JA, Omoruyi JI. Studies on the influence of various latex yield stimulants on dry rubber content and cup lump weight in *Hevea brasiliensis*.