CLONAL VARIATION IN PHLOIC RAY CHARACTERS IN THE SECONDARY PHLOEM TISSUE OF *HEVEA* CLONES

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Secondary phloem or bark, which is formed from the cambial tissue present in the peripheral side of woody plants, plays a pivotal role in the transport of photosynthetic assimilates and various other chemical molecules. In *Hevea brasiliensis*, the various cell components of bark include sieve tubes, companion cells and phloem parenchyma. The present study deals with the distribution and dimensional features of ray parenchyma in ten different clones of *H. brasiliensis*. The presence of ray was identified from soft bark region to the inner hard bark region and extended up to the outer hard bark region. The study illustrates three principal types of ray cells in the secondary phloem which are uniseriate, biseriate and multiseriate in nature. Among them, the proportion of multiseriate rays is far more when compared to uni and biseriate rays. Statistical analysis indicated significant variation among the clones for biseriate and multiseriate rays in soft bark and inner hard bark region. Significant clonal variation was observed for anatomical features such as height, width and height/width ratio of phloic rays. The variation was highly noticeable in the inner hard bark region. The characters which suggest significant clonal variation can be considered as prominent anatomical traits in breeding and exploitation strategies in *Hevea* clones. The study indicated that the frequency and dimensional features of phloem rays in the various zones of the secondary phloem were significant from the anatomical and structural perspective of the bark of *Hevea*.

Keywords: Clonal variation, *Hevea brasiliensis*, Phloic rays, Secondary phloem

INTRODUCTION

Hevea brasiliensis belonging to the family Euphorbiaceae is the major source of Natural Rubber (NR) for commercial use in the world. The secondary phloem, which is also called as bark, is the tissue system which harbours the latex vessels or laticiferous tissues which possess latex. Ten species recognized under the genus *Hevea* so far are: *H. guianensis, H. brasiliensis, H. pauciflora, H. spruceana, H. rigidifolia, H. benthamiana, H. nitida, H. microphylla, H. camporum* and *H. camargoana*, in the order of first descriptions of the concepts (Schultes, 1970; 1977; 1987; Wycherley, 1992; Varghese and Abraham, 2005). Of these species, *H. brasiliensis* contain a well-developed laticiferous system and cultivated for commercial exploitation of latex.

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Phloem is the major tissue system devoted for the transport and distribution of organic materials produced by photosynthesis to all parts of the plant body. In addition, it also serves as the system for transport of signalling molecules such as mRNAs, hormones and biomolecules of defence mechanism, storage of many ergastic molecules such as starch, calcium crystals, phenolics and tannins. The complex phloem tissue system primarily consists of sieve tubes, companion cells, parenchyma (both axial and ray) and phloem sclerenchyma. Secondary phloem which comprises the bark is the principal tissue meant for the transport of organic nutrients in tree plants (Pace, 2019).

Anatomically, the bark of *H. brasiliensis* consists of three zones, *viz.* innermost region close to cambium is known as soft bark (SB), outer to it forms the inner hard bark region (IHB) and the outer most tissue system constitute outer hard bark (OHB) region, which is mainly characterized by the nature and character of laticifers and presence of sclerified stone cells (Omman and Reghu, 2008). Ray cells in *Hevea* may have great anatomical significance as it comprises the main component of the secondary phloem. The occurrence and distribution of sieve tubes and companion cells have been recorded earlier in *Hevea* in the soft bark and inner hard bark region (Omman and Reghu, 2013a). The laticiferous tissue system is highly organized, anastomosing and placed in rows in secondary phloem (Omman and Reghu, 2013b). The present investigation is an attempt to understand various characters associated with phloem rays in the soft bark and inner bark region in ten important cultivated clones of *Hevea*. The anatomical features of uniseriate, biseriate and multiseriate phloem rays with respect to its individual frequencies, height and width showing distinct clonal variability plays a significant role in the distribution of laticifers in *Hevea*.

MATERIALS AND METHODS

Ten Wickham clones of *Hevea brasiliensis* (Willd. ex Adr de Juss.) Muell. Arg., were selected from the Germplasm gardens at the Central Experimental Station of Rubber Research Institute of India, Chethackal, Ranni, Kerala. The experimental station is situated at 9° 22' N latitude and 76° 50' E longitude with an altitude of 80 m above the MSL. Trees were planted in Randomized Block Design (RBD) with three replicates and three trees per plot. The trees were under regular tapping and had an age of 17-21 years. Nine mature trees from each clone (three trees per replication) were selected. Virgin bark samples were collected from the selected trees at 150 cm height and were fixed in Formalin-Aceto-Alcohol (FAA) and were sectioned at 30 - 60 μm thickness at Tangential Longitudinal Section (TLS) using Reichert-Jung sledge microtome. Sections were stained with Oil Red O (Omman and Reghu, 2003) for laticifers and mounted in 50 per cent glycerin. The bark sections were observed under Leitz Aristoplan Research microscope attached to Leica Q 5000 I W Image Analysis System. Photomicrographs were taken by Wild MPS 46 Photo Automat using Kodak Gold 35 mm colour film in Leitz Aristoplan Research microscope.

Phloem ray characteristics were measured at a unit distance of 765 μm. Eight phloic traits *viz.*, frequency of uni, bi and multiseriate rays contiguous to latex vessels in SB and IHB, total frequency of phloic rays contiguous to latex vessels in SB and IHB, frequency of uni, bi and multiseriate rays in latex vessel-free zone in SB and IHB, total frequency of phloic rays in latex vessel free zone in SB and IHB, height and width of phloic rays (μm) contiguous to latex vessels in SB and IHB, height and width of phloic rays (μm) in latex vessel free zone in SB and IHB, height/width ratio of phloic rays contiguous to latex vessels in SB and IHB and height/width ratio of phloic rays in latex vessel free zone in SB and IHB were recorded and analyzed.

The coefficient of variation (CV) was calculated to ascertain the tree to tree variation within clones. The CV was not calculated wherever the data was incomplete due to the absence of that particular character. The variation within trees was taken as low, medium and high for the CV values. For example, 0 to 30 was taken as low, 31 to 50 as medium and 51 and above as high. The data were subjected to analysis of variance (ANOVA) to measure the extent of clonal variation between different clones using the software SPSS 10.

RESULTS AND DISCUSSION

Ray cells are an important component of phloem elements and their presence can be observed from the near region of the cambium to outermost region of the bark. The features of the ray elements observed in the present study are described.

Frequency of uni, bi and multiseriate phloic rays contiguous to latex vessels in SB and IHB

In the SB region, the frequency of uniseriate rays was maximum in RRII 105 (1.88) and minimum in RRIM 600 (0.69) (Table 1; Fig. 1). The uniseriate rays were very few in trees of all clones and did not show any clonal variability. The frequency of biseriate rays (Table 1) was maximum in GT 1 (1.17) and the three clones *viz.* PB 86, Tjir 1 and RRII 300 had a uniform minimum frequency (0.06) of biseriate rays. Biseriate rays were absent in three clones *viz.* RRIM 600, RRII 105 and PB 235. Significant clonal variability was observed in the frequency of biseriate rays and GT 1 and PB 28/59 were superior to other clones. The maximum frequency of multiseriate rays (Table 1) was noticed in PB 235 (7.00) and minimum in RRII 105 (5.98). The frequency of multiseriate rays did not show significant clonal variation or tree-to-tree variation.

The occurrence of uniseriate rays contiguous to laticifers per unit distance was considerably low in IHB (Table 1). The uniseriate rays were found only in RRII 105, RRII 300 and GT 1. Clonal variability was not statistically significant. The frequency of biseriate rays in this region was noticed only for GT 1, PB 28/59 and RRII 105. The clonal variations for this character were also not significant. The frequency of multiseriate rays was highest in GT 1 (6.56), followed by PB 28/59 (6.23) and Tjir 1 (6.22) and minimum in Gl 1 (5.22). Tree to tree variation within clones was very low as obvious from the low CV values. None of the clones showed significant clonal variation with respect to the frequency of multiseriate rays either.

Total frequency of phloic rays contiguous to latex vessels in SB and IHB

The total frequency of phloic rays in SB zone *i.e.* the sum of uni, bi and multiseriate rays was the highest for GT 1 (8.64) and the lowest total frequency was recorded for RRIM 703 (7.39) (Table 1). Tree to tree variation within clones was low as revealed by the low CV values.

The ray frequency was considerably low in IHB region compared to SB region (Table 1). The frequency of phloic rays contiguous

Table 1. **Frequency of uni, bi and multiseriate and total rays contiguous to latex vessels per 765 μm distance in soft bark and inner hard bark**

Clones	Soft Bark						Inner Hard Bark					
	Uniseriate	Biseriate	Multiseriate		Total ray frequency		Uniseriate	Biseriate	Multiseriate		Total ray frequency	
	Mean	Mean	Mean	CV (%)	Mean	CV (%)	Mean	Mean	Mean	CV (%)	Mean	CV (%)
GI 1	0.89	0.17	6.72	22	7.78	14	0.00	0.00	5.22	11	5.22	11
GT 1	0.97	1.17	6.50	20	8.64	7	0.11	0.17	6.56	10	6.84	8
PB 235	1.17	0.00	7.00	9	8.17	9	0.00	0.00	5.78	9	5.78	9
PB 28/59	1.22	0.77	6.31	9	8.31	7	0.00	0.06	6.23	12	6.29	11
PB 86	0.72	0.06	6.72	13	7.50	6	0.00	0.00	6.00	16	6.00	16
RRII 105	1.88	0.00	5.98	11	7.86	13	0.33	0.03	6.00	11	6.36	12
RRII 300	1.47	0.06	6.89	12	8.42	5	0.17	0.00	5.75	14	5.92	10
RRIM 600	0.69	0.00	6.78	11	7.47	6	0.00	0.00	5.75	11	5.75	11
RRIM 703	0.78	0.44	6.17	12	7.39	11	0.00	0.00	5.83	15	5.83	15
Tjir 1	1.17	0.06	6.94	15	8.17	3	0.00	0.00	6.22	7	6.22	7
VR (F)	0.93	3.10	0.46		2.41		2.43	0.89	1.59		2.05	
CD (P<0.05)	NS	0.69	NS		NS		NS	NS	NS		NS	

to latex vessels in the inner hard bark zone was highest in GT 1 (6.84) and lowest in Gl 1 (5.22). Tree to tree variation was very low.

Frequency of uni, bi and multiseriate rays in latex vessel-free zone in SB and IHB

The frequency of phloic ray in latex vessel free zone is presented in Table 2. The occurrence of uniseriate rays in SB was observed in all clones except RRIM 703 and its frequency was maximum in RRII 300 (1.50) and minimum in PB 86 (0.22). However, the clonal variation was not significant as revealed by the analysis of variance. The highest frequency of biseriate rays was recorded in RRIM 703 (0.78) and the lowest in PB 86 (0.06). Such rays were absent in many of the trees and hence the tree to tree variation was not worked out. The clones RRIM 703, PB 28/59 and GT 1 were on par and they were statistically superior to the rest of the clones for this trait. The frequency of multiseriate rays was highest in Tjir 1 (7.18) and lowest in Gl 1 (5.58). Tree to tree variation was low and significant clonal variation was noticed. Tjir 1, PB 235, RRIM 600 and GT 1 are at par and superior to other clones.

The frequency of uniseriate rays was drastically low in IHB. Only two clones, GT 1 (0.22) and PB 86 (0.11) showed presence of uniseriate phloic rays in low frequency in the latex vessel free zone. The tree to tree variation and clonal variation were insignificant. None of the clones showed the presence of biseriate rays. The clonal variation was highly significant for multiseriate ray character. Clones PB 86 (6.28), GT 1 (6.11) and PB 28/59 (6.08) were the top rankers. Gl 1 recorded the minimum value (5.00). Tree to tree variation was very low.

Total frequency of phloic rays in latex vessel-free zone in SB and IHB

The total frequency of rays in the laticifer free zone in SB showed considerable variation among clones (Table 2). The highest frequency was observed in Tjir 1 (8.24) and the lowest in Gl 1 (6.16). The tree-to-tree variation within clones was very low. The clones Tjir 1, GT 1, PB 235, RRII 105, RRII 300 and RRIM 600 were comparable with respect to this character.

Significant clonal variation was observed in the total frequency in IHB also. PB 86 recorded the highest frequency (6.39) and the lowest frequency was associated with Gl 1 (5.00). None of the clones showed significant tree to tree variation. The clones PB 86, GT 1 and PB 28/59 recorded comparable total frequencies and were superior to the rest of the clones for this character.

Height, width and height/width ratio of phloic rays contiguous to latex vessels in SB and IHB

The height and width of phloic rays contiguous to latex vessels are presented in Table 3. The height of rays contiguous to laticifers in SB varied considerably in all the clones. The highest (Fig. 1a) ray height was observed in PB 235 (400.64 μm) and the lowest (Fig. 1b) in RRIM 703 (292.55 μm). Tree to tree variation was less. Analysis of variance indicated that PB 235, GT 1 and PB 86 were on par and superior to other clones.

The width of phloic rays in the SB region did not vary significantly between clones. The tree to tree variation within the clones for this character was also very low.

The height of the rays was considerably reduced in IHB. Within the clones, highest ray height was noticed for PB 86 (382.94 μm).

Table 2. **Frequency of uni, bi and multiseriate rays in latex vessel-free zone per 765 μm distance in soft bark and inner hard bark**

Clones	Soft Bark						Inner Hard Bark					
	Uniseriate	Biseriate	Multiseriate		Total ray frequency		Uniseriate	Biseriate	Multiseriate		Total ray frequency	
	Mean	Mean	Mean	CV (%)	Mean	CV (%)	Mean	Mean	Mean	CV (%)	Mean	CV (%)
Gl 1	0.58	0.00	5.58	10	6.16	11	0.00	0.00	5.00	11	5.00	11
GT 1	0.78	0.56	6.61	13	7.95	7	0.22	0.00	6.11	10	6.33	8
PB 235	1.06	0.00	6.78	12	7.84	8	0.00	0.00	5.67	16	5.67	16
PB 28/59	0.59	0.61	6.14	10	7.34	12	0.00	0.00	6.08	7	6.08	7
PB 86	0.22	0.06	6.44	8	6.72	11	0.11	0.00	6.28	17	6.39	15
RRII 105	1.15	0.00	6.28	13	7.43	13	0.00	0.00	5.16	9	5.16	9
RRII 300	1.50	0.00	5.86	16	7.36	10	0.00	0.00	5.64	11	5.64	11
RRIM 600	0.61	0.00	6.64	10	7.25	5	0.00	0.00	5.22	9	5.22	9
RRIM 703	0.00	0.78	6.36	8	7.14	11	0.00	0.00	5.47	10	5.47	10
Tjir 1	1.06	0.00	7.18	8	8.24	7	0.00	0.00	5.61	11	5.61	11
VR (F)	2.34	6.23*	3.55*		3.76*		0.87		3.93*		5.25*	
CD (P<0.05)	NS	0.38	0.73		0.86		NS		0.64		0.62	

*Significant at P<0.01

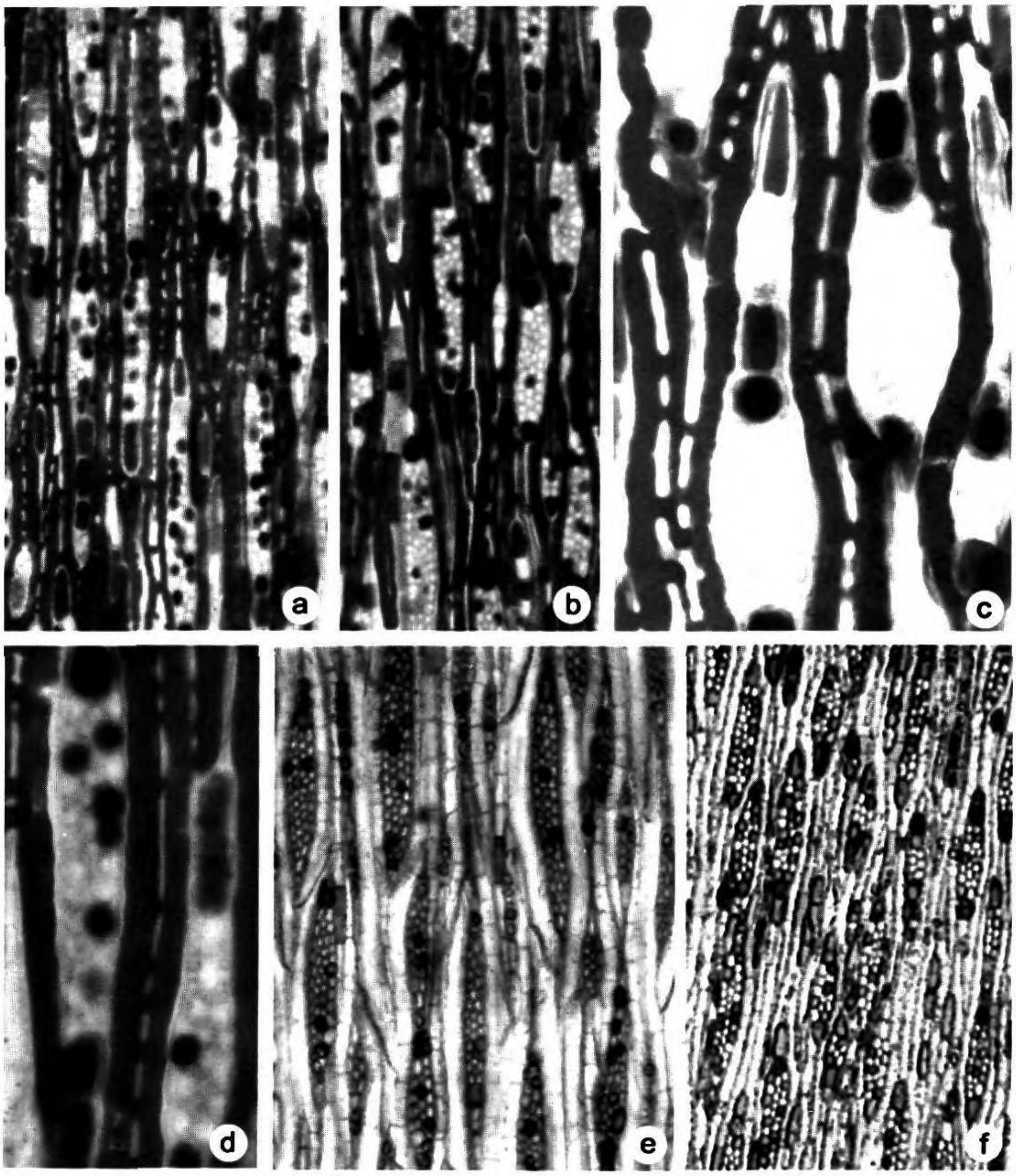

Fig. 1. TLS of bark showing height and width of phloic rays in soft and inner hard bark a-d: Contiguous to latex vessels; e & f: Latex vessel-free zone. a) PB 235 - Highest ray height in SB; b) RRIM 703- Lowest ray height in SB; c) GI 1 -Highest ray width in IHB; d) GT 1 - Lowest ray width in IHB; e) PB 235 - Highest ray height in SB; f) RRIM 703 - Lowest ray height in SB

The clones GT 1, GI 1, PB 28/59 and PB 235 also recorded comparable ray height. The ray height was the lowest in RRIM 703 (267.03 μm). Tree to tree variation for ray height within the clones was very low.

The width of phloic rays in IHB was maximum (72.85 μm) in Gl 1 (Fig. 1 c) and minimum (54.25 μm) in GT 1 (Fig. 1 d). The CV values did not depict any marked tree to tree variation. Similarly, the variation between clones was also not significant.

Significant differences were observed in the mean height/width ratio among the clones in both SB and IHB region while the tree to tree variations were low as indicated by the CV values (Table 3). The height/width ratio of the rays contiguous to laticifers was higher in SB region compared to IHB region of the bark tissue. The highest ratio was observed in SB region for PB 235 (11.09) followed by GT 1, PB 86, Tjir 1 and RRIM 300 which were comparable. The smallest ratio was recorded for RRII 105 (6.44). In the IHB region, mean height/width ratio was highest for GT 1 (6.59) followed by PB 235 (6.03) which were comparable and superior to all other clones for this trait indicating their possible association with high growth vigour.

Height, width and height/width ratio of phloic rays in latex vessel-free zone in SB and IHB

The height and width of rays in the laticifer-free zone of SB and IHB regions are presented in Table 4. In SB region, the highest ray height was recorded (Fig. 1e) in PB 235 (387.87 μm) and the lowest (Fig. 1 f) in RRIM 703 (307.30 μm). Tree to tree variation within clones was low in all the clones. Significant clonal variation was noticed. The mean height was comparable for the clones PB 235, Gl 1, PB 86 and GT 1.

Table 3. **Height, width and height/width ratio of phloic rays contiguous to latex vessels per 765 μm distance in soft bark and inner hard bark**

Clones	Soft Bark						Inner Hard Bark					
	Height (μm)		Width (μm)		H/W Ratio		Height (μm)		Width (μm)		H/W Ratio	
	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)
Gl 1	314.70	22	40.99	9	7.78	22	345.31	12	72.85	8	4.75	14
GT 1	376.04	8	36.11	19	10.50	14	354.02	11	54.25	19	6.59	23
PB 235	400.64	18	36.10	12	11.09	17	334.03	7	55.89	17	6.03	15
PB 28/59	326.26	11	39.51	18	8.42	19	335.34	16	62.64	14	5.38	17
PB 86	367.09	9	41.63	14	9.04	18	382.94	14	70.28	20	5.48	22
RRII 105	300.56	9	46.71	14	6.44	18	296.95	11	57.62	12	5.16	17
RRII 300	319.52	8	36.34	17	8.83	16	299.62	8	60.19	15	5.01	14
RRIM 600	297.08	13	40.90	11	7.26	7	293.45	9	70.27	5	4.18	9
RRIM 703	292.55	5	40.73	8	7.21	8	267.03	7	59.43	19	4.55	18
Tjir 1	318.57	17	36.55	17	8.90	21	298.26	14	61.52	9	4.85	14
VR (F)	2.45 *		2 .33		2.88 *		3.43 *		2.33		4.29 **	
CD (P<0.05)	70.20		NS		2.56		56.37		NS		1.02	

*Significant at P<0.05 **Significant at P<0.01

The width of phloic rays in SB region was highest in Gl 1 (49.39 µm) and lowest in GT 1 (34.05 µm). Clonal variability was not significant. Within the clones, tree to tree variation was low.

The height of rays in IHB region was highest in Gl 1 (380.51 µm) and lowest in RRIM 703 (277.03 µm). The tree to tree variation within the clone was relatively low. Analysis of variance revealed that the difference in ray height between clones was statistically significant where Gl 1 and PB 86 were on par and statistically superior to other clones for this trait.

The width of the rays increased in this zone with the maximum value of 78.04 µm in Gl 1 and the minimum value of 52.88 µm in GT 1. Within the clones, variations in ray width were negligible. Clonal variation for this character was statistically significant (Table 4). Clones Gl 1, RRII 105 and RRIM 600 were comparable and superior to the rest of the clones for this trait.

The height/width ratio exhibited significant clonal variation only in the IHB in latex vessel-free zone. In SB, clonal differences were not significant. The clones, GT 1 (6.17) and PB 86 (6.10) recorded comparable ratios and were significantly superior to others for this trait. In SB as well as IHB, the tree to tree variations were comparatively less as indicated by the low CV values.

The principal phloic ray types observed in *Hevea* are uniseriate, biseriate and multiseriate, of which multiseriate rays are the most abundant (about 95-98%). The occurrence of more multiseriate rays in the secondary phloem has also been reported in many other genera (Den Outer, 1986; Varma *et al.*, 1993; Lu *et al.*, 1994; Liu, *et al.*, 1995; Heo, 1996; Carlquist, 1999; 2000). The clonal

Table 4. **Height, width and height/width ratio of phloic rays in latex vessel-free zone in soft bark and inner hard bark**

Clones	Soft Bark						Inner Hard Bark					
	Height (µm)		Width (µm)		H/W Ratio		Height (µm)		Width (µm)		H/W Ratio	
	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)	Mean	CV(%)
Gl 1	381.65	8	49.39	16	7.85	18	380.51	11	78.04	9	4.88	16
GT 1	351.57	15	34.05	14	10.33	11	326.37	15	52.88	15	6.17	19
PB 235	387.87	16	43.96	17	9.48	21	315.66	11	66.63	9	4.73	9
PB 28/59	320.95	9	38.80	16	8.34	19	324.80	9	67.78	12	4.81	17
PB 86	380.36	11	40.88	20	9.53	22	344.74	14	57.13	21	6.10	23
RRII 105	315.79	11	45.82	14	7.04	13	287.06	12	74.54	11	3.86	16
RRII 300	321.68	22	35.47	16	9.10	24	287.25	7	67.76	21	4.30	24
RRIM 600	326.74	10	43.44	12	7.57	12	293.45	4	70.27	12	4.18	11
RRIM 703	307.30	4	40.73	23	7.61	18	277.03	8	62.77	17	4.44	19
Tjir 1	318.84	14	36.00	15	8.86	18	315.82	12	64.36	8	4.93	12
VR (F)	4.42 **		1.30		1.59		3.19 *		5.03 **		5.26 **	
CD (P<0.05)	44.08		NS		NS		52.22		9.94		0.99	

* Significant at P<0.05; ** Significant at P<0.01

variation in ray morphology contiguous to latex vessels was not significant whereas significant variation in ray morphology was observed in those rays present in the latex vessel free zone. The total frequency of phloic rays was more in the soft bark region than in the hard bark region. Similarly, frequency of multiseriate rays increased in the inner hard bark region compared to uni and biseriate rays. This increase may be due to the conversion of uni and biseriate rays to multiseriate rays during the transition of soft bark to inner hard bark. Even though the frequency of different ray types and total frequency do not possess any clonal variation in the rays contiguous to laticifers zone in SB, the same exhibited significant variation in latex vessel-free zone in SB and IHB regions. This information derived from the present study is a novel finding on the anatomical characterization of *Hevea*. Regarding total ray frequency, generally RRIM 600 and RRII 105 clones showed less clonal superiority over other clones and PB clones recorded higher ray frequency.

Phloic rays run radially in the bark tissue and have a great physiological role in the conduction of materials especially of laticifers (Hebant and De Fay, 1980). The height of rays in most of the clones, was more in the soft bark than the inner hard bark, whereas the ray width showed a reverse trend. This may either be due to dilation of cells of the rays during the transition of soft bark to inner hard bark or due to the fusion of ray groups as reported in Oak species (Trockenbrodt, 1994). Premakumari *et al.* (1984) reported a negative correlation of ray width with latex vessel density. Significant increase in ray height in drought-tolerant clones have been reported by Premakumari *et al.* (1993). Ray height has been identified as a distinguishable character in various anatomical investigations, especially for the classification of different species within the genera (Magistris and Castro, 2001). Significant clonal variability in the height/width ratio of phloic rays between virgin bark and renewed bark has been reported earlier (Premakumari *et al.*, 1992). Thomas *et al.* (2013) reported that the ray length can be reduced and ray width may be increased in trees affected with tapping panel dryness (TPD), in comparison with healthy trees in *H. brasiliensis*.

The reduction in H/W ratio of phloic rays in the inner hard bark zone may be due to increase in the width of rays in this zone. The occurrence of highest H/W ratio in GT 1 compared to other clones investigated is a noteworthy observation. The height and height/width ratio exhibited significant clonal variation as seen in GT 1 and PB 86. It has been reported that ray width was negatively associated with the density of laticifers and this association had a direct influence on the running direction of latex vessels (Premakumari *et al.*, 1985; 1988). The in-depth findings of the present investigation could be utilized in clonal selection strategies for choosing desirable structural traits.

CONCLUSION

Phloic rays are one of the important components among the phloic derivatives present in the bark tissue system of *Hevea brasiliensis*. Phloic rays in the mature trees were identified as uniseriate, biseriate and multiseriate. Multiseriate rays are the major category followed by biseriate and uniseriate types. Their presence was distinguished from the SB region to the peripheral IHB and OHB areas in the radial direction. Major clonal variation was observed in the ray types and the total number of phloic rays. The height, width and height/width ratio also exhibited significant clonal variation. The outcome of the present anatomical investigation can be utilised further for identifying secondary traits connected with functional active tissues.

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