

Radial variation of wood cell features under different stocking densities management of two new clones of rubberwood (*Hevea brasiliensis*)

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Abstract Wood characteristics vary from pith to bark and this can influence the final use. These variations can be related to the effects of plantation management. The radial variation in fiber cells, vessel elements, and ray cells was investigated for a commercial and fast-grown species, the rubberwood (*Hevea brasiliensis*), at varying stocking densities. Nine-year-old trees of two new clones (RRIM 2020 and RRIM 2025) were categorized into four stocking densities of 500, 1000, 1500, and 2000 trees ha⁻¹. The sample blocks were anatomically analyzed to determine the changes in wood cells from pith to bark. The results generally showed a centrifugal increase in fiber features, vessel diameter, ray height, and ray area. Vessel density and ray density showed a decreasing trend. Vessel areas revealed a radial irregular variation. The ray area showed a striking relationship with ray density and ray height. Ray cell pattern indicated enough variation for visual approval to

ensure within and between stocking density changes. Most radial variation was explained by the effect of cambial age. Owing to different stem width and growth rates, the mean cell features were not at the same radial distances from the pith.

Keywords Radial variation · Anatomical properties · Stocking density · Clonal effect · Rubberwood

Introduction

Rubberwood (*Hevea brasiliensis* Muell. Arg.) is presently widely planted in Malaysia (over 1.5 million ha) to produce rubber (as an industrial raw material) and increase the supply of timber so as to decrease the exploitation of Malaysian natural jungles [1–3]. Some 80 % of the Malaysian furniture content is derived from rubberwood products either in the form of solid timber or reconstituted panel products [4]. To establish plantations of rubberwood species, we need to know the wood properties among the tree varieties. Especially, in tropical fast-grown plantations, attention is focused on the association between radial growth rates and wood quality of xylem yield [2]. The wood properties analyzed transversely across the radial direction, around the bark, or from stem base upwards [2, 5] and this variability has a significant influence on the final use of the wood [6, 7]. A comprehensive understanding of the sources of variation in the structural characteristics of the wood due to effects of plantation management practices is necessary. One approach toward this understanding is to study the variation in wood structure due to different stocking densities. Dodd [8] observed variation in wood structure within the tree in relation to the crown in sycamore (*Acer pseudoplatanus*). To the best of the authors'

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knowledge, there is no specific study on the effect of different stocking densities on the radial variation in rubberwood structure. Lim and Fujiwara [9] had investigated variation in wood density in two clones of rubberwood at three different planting densities. The radial variation in wood anatomical characteristics may have an important effect on the quality in terms of processing and product manufacture [10]. To realize the optimum utilization of these timber species, a detailed study of the radial variation of wood features is required. Muhammed and Sahri [11] and Norul Izani and Sahri [12] had reported an increasing trend for fiber and vessel cell anatomical properties from pith outwards in different clones of rubberwood. The present study investigates on sizes and amounts of variation in the radial direction for fibers, vessels, and ray cells of rubberwood trees planted at four different densities.

The objective of this study was to determine the radial wood anatomical properties of plantation-grown *Hevea brasiliensis* in Malaysia for rational utilization of the wood. The investigation was centered on radial variations in fibers, vessels, and ray cell features as influenced by planting density.

Materials and methods

Samples were collected from two new rubberwood clones namely clone RRIM 2020 (clone I) and RRIM 2025 (clone II) with four different stocking densities: 500 (SD I), 1000 (SD II), 1500 (SD III), and 2000 (SD IV) trees ha⁻¹ growing in a 9-year-old plantation plots. These plots at a latitude of 5°45'0"N and longitude of 102°30'0"E are managed by the Malaysian Rubber Board in the northeastern part of Peninsular Malaysia. The average precipitation during the last 3 years was recorded at 3752 mm [13].

The main criterion in these plots was stocking density (SD, i.e., number of tree per hectare). This plantation was established in year 2000 to determine the effects of spacing on wood quality. All plots were under natural conditions and located close by to each other. Nearly 1 ha was assigned to each stocking density. Tree samples were selected from even stands (Table 1), and trees growing adjacent to the roadside or near large open spaces and inclined trees were avoided. All trees sampled had fairly straight stems, were not defective and were growing on a relatively uniform terrain. In addition, no any tapping process was done on the trees.

The trial was carried out in a randomized complete block design. Two trees from each stocking density with a total of sixteen trees from the two clones were sampled. The trees were cut at a height of 15 cm above the ground level. A cross-sectional disc of approximately 50 mm in thickness collected from each tree at 1.3 m above ground level was used in the determination of radial anatomical component characteristics. The discs were labeled, wrapped in black plastic bags and transported to the laboratory of wood anatomy for further analyses. Owing to indistinct growth rings in the samples, the radial variation in fibers, vessels, and wood ray cells for each disc was analyzed on a strip running from pith to bark [14, 15]. Radial blocks with precise dimensions of 15 × 15 × 15 mm with no intervals between them were cut from each strip. After removing the pith, 4 blocks from SD I and 3 blocks from SDs II, III, and IV were prepared, depending on the diameter at breast height of sampled trees and the radius of the strips. These blocks would enable detection of the gradual radial change in wood anatomical features. All blocks were properly numbered. Almost the middle part of each block was chosen for sectioning. Transverse and tangential micro-sections with thicknesses of 20–25 μm were obtained from

Table 1 Basic information of the *H. brasiliensis* plantation

Clone ^a	No. of trees (trees ha ⁻¹)	Stocking density	Stocking distance (m)	DBH ^b Mean (cm)	BH ^c Mean (cm)
I	500	I	4.0 × 5.0	20.22 (3.88)	467 (107)
	1000	II	4.0 × 2.5	19.19 (3.20)	863 (100)
	1500	III	3.0 × 2.2	17.43 (3.74)	1023 (130)
	2000	IV	2.0 × 2.5	17.54 (3.91)	936 (110)
II	500	I	4.0 × 5.0	19.96 (2.29)	738 (121)
	1000	II	4.0 × 2.5	16.29 (2.28)	788 (77)
	1500	III	3.0 × 2.2	15.27 (2.36)	909 (98)
	2000	IV	2.0 × 2.5	15.07 (2.38)	1026 (107)

Values within brackets are standard deviations

^a Clone I = RRIM 2020 and clone II = RRIM 2025

^b Over bark values

^c BH = bole height

each small block using a sledge microtome. To determine the fiber cell features, the maceration process used followed the TAPPI Standard T233-Su-64 [16]. The fiber length (μm), fiber diameter (μm), fiber lumen diameter (μm), and fiber wall thickness (μm) of randomly selected fibers were measured by image processing software calibrated with a stage micrometer. Fiber diameter, fiber lumen diameter and fiber wall thickness were measured with a magnification of $\times 400$ and for fiber length, vessel and ray cell components, a magnification of $\times 100$ was used. A preliminary study was conducted to determine the number of fibers to be measured. High values of standard deviations were obtained with <30 readings for each of the features recorded. Therefore, the final countable number was increased to 50 readings for every single component. Crosswise sectional microscopic images were measured using an Image Analyzer Microscope (Leica Qwin model). The vessel density (mm^{-2}), vessel diameter (the average of radial and tangential diameter, μm), and vessel area (%) were determined in every field under microscope. Likewise, ray density (mm^{-2}), ray height (number of parenchyma cells in every single ray cell), and ray area (tangential surface occupied by ray cells, %) were calculated in tangential section.

The statistical analyses on mean differences in radial direction at each SD of clones were tested using the statistical package for Social Science (PASW[®] statistics processor, version 18). The data were subjected to an analysis of variance procedure to examine variability in the various wood anatomical properties. The Duncan Multiple Range Test (DMRT) was used to separate the means of the various growth categories at 5 % probability.

Results

Fiber size

The radial variation in the fiber length of the four SDs from the two clones is presented in Fig. 1a. The fiber length demonstrated a similar pattern with a gradual radial increase, from pith to bark, for both clones irrespective of SD. The fiber lengths in SDs I from the two clones also showed an amazingly different pattern in which the fiber lengths increased from 1148 μm and 1217 μm (near the pith) to 1332 μm and 1457 μm (the second to last specimen near the bark) and then decreased to 1279 μm and 1438 μm in last specimen near the bark, respectively. This showed a fluctuated pattern in radial direction. SD IV from clone I and SD III from clone II showed the highest increase (39.1 and 32.7 %, respectively) in fiber length near the bark compared to fiber length near the pith.

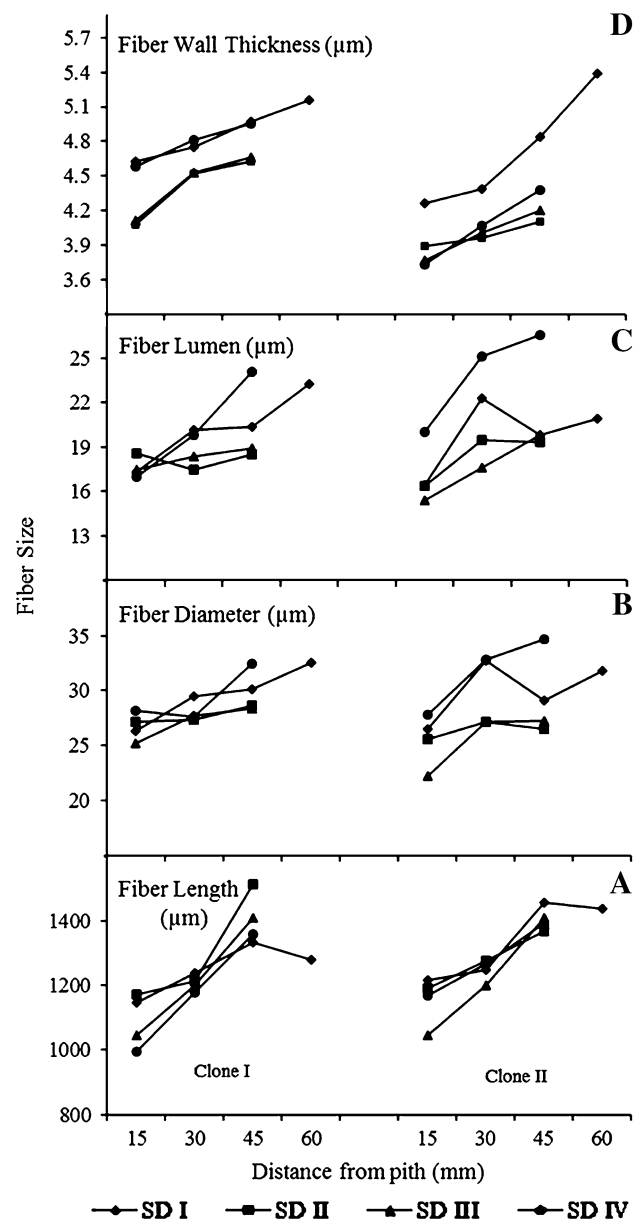


Fig. 1 Variations in mean fiber length (a), fiber diameter (b), fiber lumen (c), and fiber wall thickness (d) with the distance from the pith at different stocking densities of rubberwood (see legends). Each panel represents the data of two clones in left- and right-hand sides

Fiber diameter showed a pronounced ascending trend from pith to bark in all SDs except for SD IV in clone I which showed a slight decrease in sample II (30 mm far from the pith) and sample I (15 mm far from the pith) of clone II showing a swing movement (Fig. 1b). Fiber diameters in all SDs from clone I showed 23.6, 5.4, 12.7 and 15.1 % increase, while clone II showed 23.4, 3.9, 22.2 and 24.9 % increase between the highest and lowest values, respectively.

Fiber lumen diameter in SD II from clone I and SD I from clone II did not show a constant pattern, while the rest represented a radially increasing trend (Fig. 1c). In clones I and II, the trend showed a change of 34.2, -0.5, 8.4, 41.6 %; and 27.5, 18.2, 28.8, and 32.7 %, respectively.

The fiber wall thickness from the pith to the bark at all SDs of the two clones demonstrated a similar increasing trend (Fig. 1d). The fiber wall thickness was shortest near the pith and increased toward the periphery, reaching its maximum near the bark. Figure 1d shows significant increases of 11.5, 8.1, 13.4, and 13.5 % for clone I and 26.5, 17.4, 11.4, and 5.4 % increases for clone II comparing between the first and last specimens, respectively.

Vessel anatomy

The pattern of vessel density (mm^{-2}) variation showed approximately a similar decreasing trend from the pith to the bark for all SDs of both clones except for SD II of clone I with no significant differences between them.

The manner in which the vessel density decreases with increasing distance from the pith within individual SD is

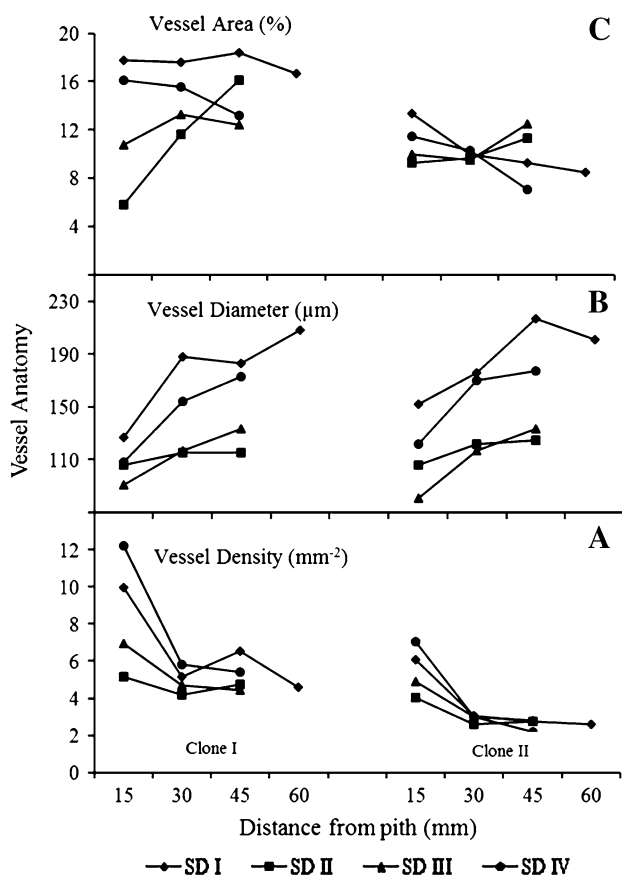


Fig. 2 Variations in mean vessel density (a), vessel diameter (b), and vessel area (c) with distance from pith at different stocking densities of rubberwood

illustrated in Fig. 2a. The magnitude of variation was different for different SD and clones. A significant -53.9, -7.0, -2.6, and -58.1 % change in clone I; and -57.2, -31.3, -42.9, and -68.9 % change in clone II were recorded for vessel densities near the bark compared to near the pith, respectively.

The mean vessel diameter (μm) was materially larger in samples near the bark than those near the pith; and both clones followed a constant pattern and increased almost linearly (Fig. 2b). A pith-to-bark trend showed an increase of 62.5, 8.5, 46.2 and 60.2 % in clone I and 32.2, 17.9, 64.2, and 45.1 % in clone II, respectively. Figure 3a and b shows the difference between vessel density and diameter near the pith comparing near the bark.

The mean vessel area (the area of wood under vessel coverage, μm^2) did not exhibit a uniform pattern or a linear tendency from the pith to the bark. The graphs of vessel area versus distance from pith are presented in Fig. 2c. The degrees of variations were different for different SD and clones. In clone I by increasing SD, radial changes (%) between the first sample near the pith and last sample near the bark were -6.2, 177.6, 14.8, and -18.0 %. In clone II, the corresponding values were -36.6, 21.5, 25.0, and 38.3 %, respectively.

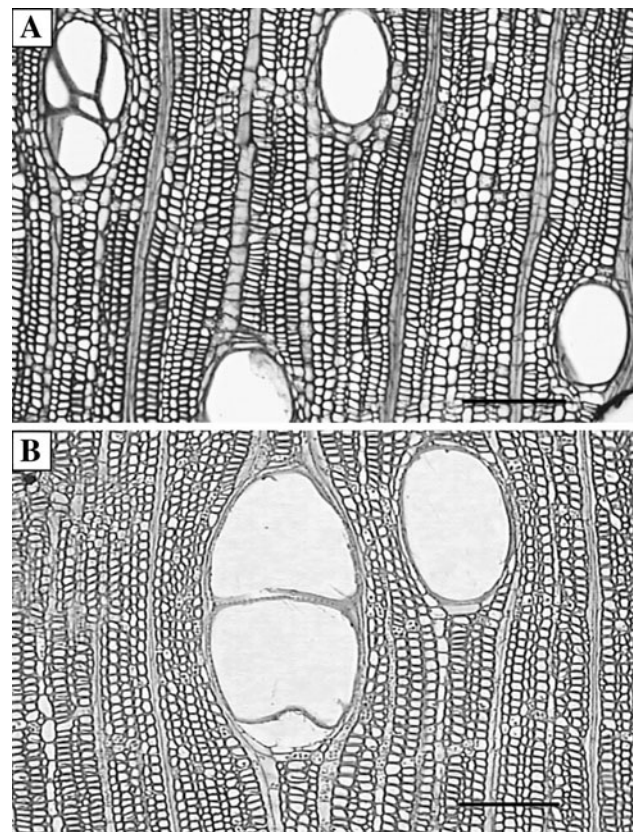


Fig. 3 Microscopic image from transverse wood sections of clone II SD II, showing the vessel density and diameter in a near the pith and b near the bark (scale bar 200 μm)

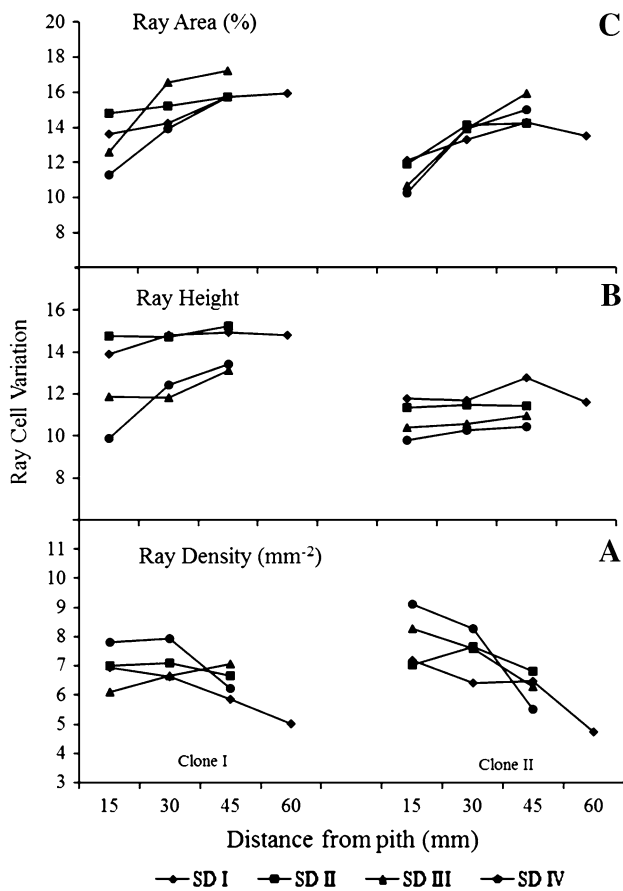


Fig. 4 Variations in mean ray density (a), ray height (b), and ray area (c) with the distance from the pith at different stocking densities of rubberwood

Ray cell variation

Very little has been published on ray cell characteristics, particularly in the radial direction. Much of the focus has been on the fiber and vessel features. To the best of the authors' knowledge, no previous reports on radial variation in ray cells of rubberwood clones and species are available.

The patterns of radial variation in ray density (mm^{-2}) in both clones were similar, except for SD III in clone I. In spite of this, no significant difference was revealed between the values of the blocks. The radial pattern showed a descending trend from the pith up to the bark (Fig. 4a). The largest values were recorded near the pith of SD IV (7.80 and 9.10 mm^{-2}) and smallest were recorded near the bark of SDs I and II (5.03 , 7.03 mm^{-2}), respectively. A pith-to-bark tendency demonstrated a change in the order of -37.8 , -5.0 , 13.7 , -25.2 %; and -52.2 , -2.9 , -31.3 , and -64.6 %, for the two clones, respectively. The highest differences (%) between first and last samples were observed in SD I and highest in SD IV.

The variations in ray height (the number of parenchyma cells in a single ray) with distance from the pith in clones I

and II are illustrated in Fig. 4b. The shortest rays were observed near the pith with an increasing trend toward the bark. The height of the rays in both clones was variable from a minimum value of 9.87 and 9.81 to a maximum of 15.22 and 12.78 , respectively.

The ray cell characteristics varied in radial direction from pith toward bark and this is strongly related to different growth rates of the clones or species [17, 18]. Consequently, in clones I and II, the changes from near the pith to the bark specimens were observed to be 6.6 , 3.1 , 10.6 , 36.1 %; and -1.7 , 0.7 , 5.2 , 6.7 %, respectively. The statistical analysis showed no significant differences between the stocking densities except for SD IV in clone I ($p < 0.05$).

The radial trend of variations in ray area did not differ much within a clone. The ray area of the *H. brasiliensis* blocks showed an increasing tendency from pith toward bark at all SDs of the clones except for SD I of clone II showing a slight decrease near the bark with no significant difference between them (Fig. 4c). The degree of variance (%) between the first sample near the pith and last sample near the bark in clones I and II was 21.5 , 6.1 , 36.8 , 39.3 %; and 21.5 , 6.1 , 28.8 , 39.3 %, respectively. These results showed that SD IV and SD II had the highest and lowest degree of variability. There were no significant differences between SDs I and II of clone I and SD II of clone II ($p < 0.05$).

Figure 5a and b represents the differences between the ray density and height in the blocks near the pith against near the bark in clone I and SD II.

Discussion

In general, fiber length of rubberwood varies from 1100 to $1780 \mu\text{m}$ [3]. The pith-to-bark increasing pattern in fiber length of *H. brasiliensis* specimens in this study was supported by several other reports [11, 12]. A slight decrease in near the bark fiber length of specimens at SD I of both clones might be associated with the early maturation of trees [7, 19–23].

Similar differences (%) between the first and the last specimens were sometimes detected up to 79 % in some species [19]. Radial variation may be more pronounced due to the effect of cambial age on the fiber cell size. A similar pattern of variation has been repeatedly reported in poplars and their hybrids, natural hybrid clones of *Acacia*, *Eucalyptus globulus* and a few other tropical trees [21, 24, 25]. In any cross-sectional profile, it is obvious that the early wood fibers laid besides the pith are much short and the next fibers are markedly longer [19, 23]. Most of the published literatures have reported that shorter fibers occur near the pith of the tree with a slight increase toward the

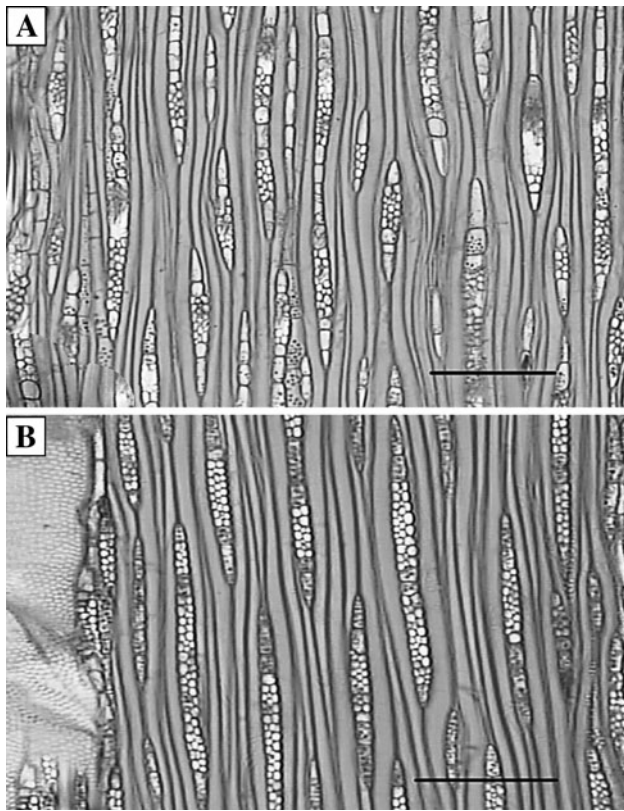


Fig. 5 Microscopic image from tangential wood sections of clone I SD II, showing the ray density and height in **a** near the pith and **b** near the bark (scale bar 200 μm)

periphery [5, 15, 19, 20, 26]. This fact is primarily related to the amount of auxin originating in young leaves that induced very short fibers [19, 27]. The analysis of variance showed significant differences between samples close to the pith versus samples close to the bark ($p < 0.05$).

In the present study, the mean diameter of wood fibers almost dropped between the ranges (23–30 μm) stated in previous reports [3, 12], while in the case of SD IV of clone II, the value exceeded this range (31.77 μm). The lowest values of average of wood fiber diameter were detected in SD II and SD III from both clones. The data showed significant differences in radial fiber diameters ($p < 0.05$) between each SD except SD II of both clones.

The mean fiber lumen diameter showed almost a constant increasing pattern, except for SD II of clone I that followed a different radial variation. The results of the mean fiber lumen diameter in this study (17.58–23.90 μm) were not comparable to those (10–12 μm) reported by Norul Izani and Sahri [12]. The maximum effect of SD on fiber lumen diameter was recorded at SD IV which showed the widest difference between samples near the pith and the bark. There was a significant difference between the lowest and highest values (pith side and bark side) for SDs I and IV of clone I and all SDs of clone II ($p < 0.05$).

The cell wall thickness of wood fibers showed different degrees of impressibility with SDs that were remarkably different and do not follow a constant pattern (Fig. 1d). The mean fiber wall thickness in rubber trees reported by Norul Izani and Sahri [12] and Teoh et al. [3] ranged from 5.00 to 7.00 μm , which were a little higher than the results recorded in this study. Wall thickness is one of the cell dimension features which is important for the final product value [28]. The radially increasing pattern due to stocking density in this study was similar to those reported in other species of rubberwood as well as other hardwoods like, black willow, sycamore, pecan, eucalypt, and sugarberry [2, 12]. The results of analysis of variance showed no significant differences between all sampled blocks in SD IV from clone I and clone II. Fiber wall thickness is of very important factor for determining wood property.

The vessel density in the present study ranged from 2.20 to 12.23 mm^{-2} that was remarkably larger than the mean vessel density of 2–4 mm^{-2} reported for rubberwood [1, 12, 29] and much closer to the findings of Syeed Saifulazry [30].

A decrease in the radius for vessel density was seen in five different species of *Hevea* [12]. Differences in magnitude between the first and last specimens at all SDs were more apparent when comparing the near pith with the near bark samples. Between SDs, the variability in clones I and II was more marked at the pith side of SD IV. In general, SD IV seems to be highly different. The profile of radial variation in vessel density (example of SD III of clone I) showed a decreasing trend from pith to bark (Fig. 6a). In addition to this variability, a significant amount of high-fluctuation variability is represented by a number of up and down swings on the graph. There were significant differences between sampled blocks near the pith versus sampled blocks near the bark in each SD of both clones, except for the SD II of clone I ($p < 0.05$).

The increase of vessel diameter with the radius for all SDs in both the rubberwood clones in this study was supported by the findings of Syeed Saifulazry [30] and Norul Izani and Sahri [12]. Likewise, similar observations were recorded in some other tropical hardwoods [2, 5, 25]. This kind of pattern can be directly related to cambial age in temperate species and radial growth increment in tropical species. The decrease in vessel density specifies a strong decrease in the number of distally functional conduits, in cooperation with a narrower diameter. The core reason for the changing vessel size along the width of the stem can be related to the fact that the vessels are induced by the polar movement of auxin hormones produced in young leaves. The higher hormone concentrations induces fast cell differentiation; while low levels stimulate slow differentiation, which allows more time for cell development until secondary wall deposition, consequently resulting in wider vessels [27, 31].

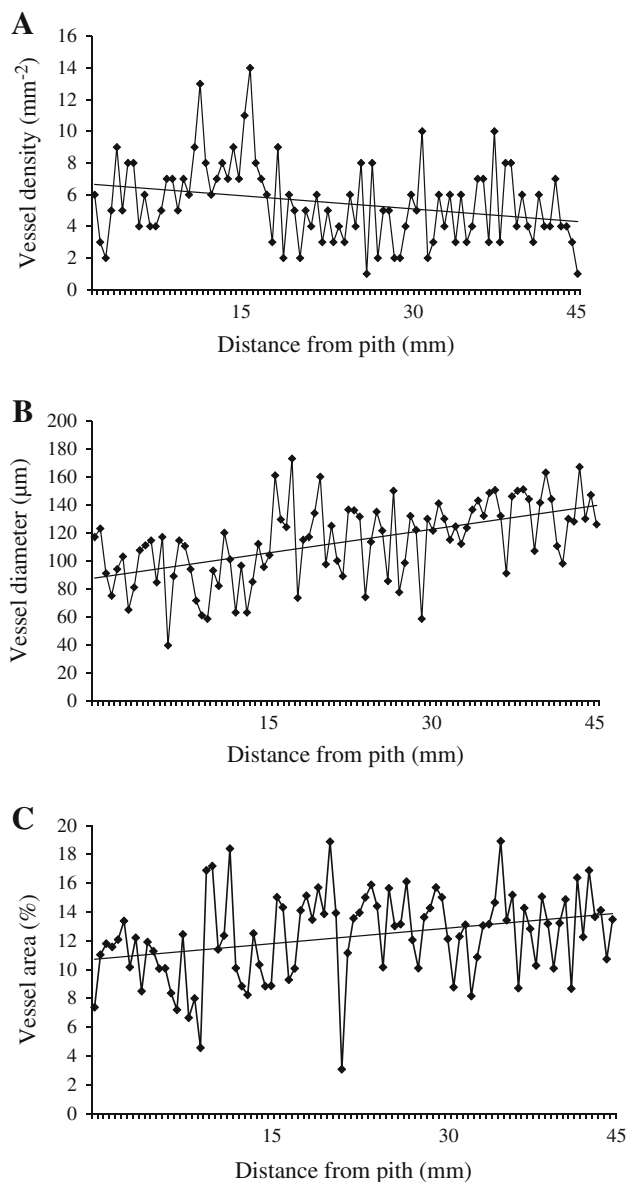


Fig. 6 Radial profile of cross-sectional vessel variations for SD III of clone I: **a** vessel density; **b** vessel diameter; and **c** vessel area showing linear trend line. The numbers in X axis indicate the samples distances from the pith

Hence, it is largely known that environmental factors influence vessel size [32]. The radial variation profile of vessel diameter (for example at SD III for clone I) represented an increasing radial trend (Fig. 6b). The analysis of variance revealed a significant difference between the lowest and highest values (pith side and bark side) at SDs I, III, and IV for clone I and all SDs for clone II ($p < 0.05$).

The remarkable differences in vessel area are related to density of vessels along with the pore size that directly affects the vessel area. Cambial activity and successive formation of vessels are an expression of a compound interaction of internal and external aspects [32]. Lachenbruch et al. [33] stated that

‘the vertical pattern of wood properties (from the tip downward to the base) is similar to the radial pattern (from the pith outward)’, and consequently auxin controls the size and density of vessels along the plant axis (or plant width) and a considerable gradient in vessel size is stimulated by the hormonal signal from the point of its function in the direction of the roots, exhibited by a change from abundant narrow vessels to fewer and wider ones. Figure 6c shows the radial profile of ray area in SD III of clone I. The analysis of data for clone I showed no significant differences between vessel areas at all SDs, except for SD II ($p < 0.05$). In clone II, the results varied considerably from clone I, with significant differences between vessel areas at all SDs, except SD II ($p < 0.05$).

The increasing trend in ray density (mm^{-2}) is characteristic of early stages of radial stem increment, and the number of rays per surface area was largest in the early rings closer to the pith, which then decreased with increasing distance from the pith until being leveled off [17, 18, 34]. This finding may be related to the adverse effect of growth rate on ray numbers [17]. The analysis of variance showed that most of the variability in ray density may be explained by cambial age which showed a linear change with radial significant differences, except for SDs II in clones I and II ($p < 0.05$).

The common pattern of maturation in woody plants is a regular enlargement in ray size with age and with distance from the pith follows a general increase in the size of other wood cells, which gradually grow to be longer and wider with distance from the stem and the stem apex [35]. The general trend in ray height showed a more or less radially ascending trend within individual SD excluding SD I from both clones. Similar results were reported by Taylor [17] and Rahman et al. [18]. Likewise, the size of ray cells measured in *Acer saccharum*, *Eucalyptus globules* also showed the same pattern [35, 36]. This increasing tendency may relate to a reducing flow of auxin concentrations from early stages of growth that leads to an increase in ray cell size [27].

Variation in ray area is considered an important factor in determining wood quality characteristics [18]. In general, the pattern showed a radially increasing linear trend. These findings were confirmed by Ohbayashi and Shiokura [37] who observed that ray area (%) was smallest near the pith and increased toward the bark.

The degree of variation was strongly influenced by the effect of SD and there were differences between SDs for the rubberwood clones. Variation in ray area was dependent on the variation in ray density and ray size (ray height). If the rate of increase in ray size (height) and the rate of decrease in ray density are stabilized, or both ray size and ray density are balanced, the ray area reaches a constant [34].

The radial variation of ray area is related to the distance from the pith and cambial age. It is believed that the ray

area has a tendency to increase in juvenile wood and reached maturity at SD I. This idea is supported by the number and slight changes in ray area values in the specimens near the bark of SDs I in the two clones, while fluctuations in fiber length at the same distance from the pith increases the possibility of this finding. The fluctuation in ray area at SD I of clone II was due to an imbalance between the rate of increase in ray height and the rate of decrease in the ray density in the samples near the bark.

Conclusion

The results showed a wide range of variation in wood cells. The attention on radial variability can be looked into as a suitable foundation owing to the large quantity of information available. It would be of economic importance if the variations can be manipulated with beneficial results.

Noticeable increases/decreases in the fiber, vessel, and ray cell components moving from pith to bark in all stocking densities were found, although some features swung radially. In the case of fiber length in SD I of both clones, a slight decrease in the last sample near the bark may relate to early maturation of the trees.

The vessel density showed a radially declining trend. The high number of vessels observed near the pith at crowded densities (SD IV) may relate to the amount of auxin hormone in those locations.

Vessel areas followed no specific variation in radial direction and were not related to cambial age.

Ray density was represented with highest number near the pith and tended to decrease toward the bark at all densities, except for SD III in clone I which showed an inverse tendency. The ray area values are directly influenced by ray density and ray size. Ray densities are visibly lesser near the bark, but having a large ray will change the ray area values. The results suggest that most of the variability in radial direction at different stocking densities is determined by the radial growth increment (DBH). Due to different DBH in different stocking densities, the maximum and minimum values of variables cannot be recorded at equal radial distances from the pith.

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