Does a Graft Located in the Canopy of a Rubber Tree Affect the Morphologies of Cells in the Adjacent wood?

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The objective of this study was to characterize the wood anatomical structure of a rubber tree clone, under the influence of two different canopy grafts. The following rubber trees were selected in the system of a double-grafted PB 311 + FX 2784 and PB 311 + MDF 180. For each tree, discs of wood were cut from the affected branch immediately below the insertion of clone at right angles to the axis, from which the regions corresponding to tension, in opposite and normal wood, were identified. The anatomical analyses were conducted in accordance with the standards established by the International Association of Wood Anatomy Committee. The Kruskal-Wallis nonparametric test was applied for multiple comparisons among the types of woods and radial positions studied, at 5% of significance. Still, multivariate associations were assessed among the anatomical characteristics of both double-grafted rubber trees, by means of a two-step cluster analysis. Quantitative morphological differences were observed in the wood cells of the double-grafted studied clones. The ray height and the vessels diameter were the most important morphologic characteristics for the distinction. The canopy clone exhibited the ability to modulate the quantitative anatomical characters of the panel clone, depending on the plant's needs.

Keywords: Canopy graft; Tension wood; Wood anatomy; Rubber tree; Radial variation

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INTRODUCTION

The rubber tree (*Hevea brasiliensis* (Willd. ex Adr Jussieu) Muell. Arg.) is a native species of the Amazon rainforest, and its economic exploitation is based on the latex obtained, starting at 5 to 7 years and extending for 25 to 30 years (Rahman *et al.* 2013). The bleeding technique improvement and the use of clones has enabled the rubber tree planting to be successful in the market, guaranteeing the planting uniformity regarding the vigor, bark thickness, latex production and properties, nutrition, and tolerance to diseases (Gonçalves and Marques 2008).

The proliferation of the fungus causing the "South American leaf blight" (*Microcyclus ulei* (P. Henn) v. Arx) in the areas of naturally occurring rubber trees motivated the double-grafting development, that is, formed by three distinct genetic materials, by means of the double-grafting technique (Moraes *et al.* 2013). The double-grafting technique consists of initially performing a basal grafting using a high productivity latex clone (panel clone), from which bleeding panels (for latex extraction) will be established, and a material of broad genetic base, originating from seeds, as rootstock. Subsequently, between eight and twelve months after the first graft, a second graft is performed using a "South American leaf blight" resistant material (canopy clone) replacing the canopy of disease-susceptible clones. Thus, it was sought to gather in the same material, high productivity aspects of latex and the resistance/tolerance to the South American leaf blight. The research with double-grafted rubber trees were directed to the performance evaluation of these trees in terms of vigor, growth, and the grafting success, aiming to select clones compatible among themselves (Miranda 2000; Moraes 2000; Moraes and Moraes 2004; Moraes *et al.* 2011, 2013). However, the effect of canopy grafting on the anatomical structure of the panel clone has not been addressed in the literature, justifying the relevance of this study.

The wood cell morphology of tree species may be changed in the light of structural adjustments arising from the plant's needs, environmental conditions, and silvicultural treatment (Naji *et al.* 2011, 2013a; Rita *et al.* 2015). This response sensitivity is responsible for inter- and intraspecific wood variation. Studies with different combinations of canopy/panel showed that the canopy graft can modify the latex characteristic of the panel clone (Moraes and Moraes 2004). This finding, associated with the structural plasticity of plant species, promoted the following question development "Do canopy grafts change the cell morphology of the panel clone wood?".

In Brazil, at the end of the latex extraction period, the rubber tree wood is commonly intended to be used in the energy sectors due to, among other factors, to its low natural durability. However, with proper treatment, the same wood presents potential for use in the furniture industry. Nevertheless, the use of wood for nobler purposes can be affected due to the recurrent presence of reaction wood (Ratnasingam and Ma 2015), the development of which is associated with the cambium uneven growth, changes in the xylem cell morphology, and chemical and ultrastructural changes of the fiber's secondary wall (Mellerowicz and Sundberg 2008). In hardwood, this wood is called tension wood, once it is formed on the side where the tension forces are requested in the wood (upper side to the stem inclination). The formation of this type of wood is associated with the mechanical and environmental tensions, such as the winds action, topography, and the canopy asymmetry (Ruelle 2014).

From macroscopic analysis, the reaction wood was observed by the pith displacement in relation to the geometric center of the log (Déjardin *et al.* 2010; Sultana *et al.* 2010) and the more widely spaced growth rings (Hillis *et al.* 2004). In freshly cut timber, the tension wood can be highlighted by a clear and bright coloration (Badia *et al.* 2005; Vidaurre *et al.* 2013).

In microscopic analysis, it is possible to observe changes in the vessel size and number, which are reduced in the tension wood region (Hiraiwa *et al.* 2007). However, for some species, the main anatomical differences are related to the fibers, which have an internal gelatinous layer (G layer) formed during the cell wall development of the fibers (Clair *et al.* 2011). This extra layer contains high levels of highly crystalline cellulose (approximately 10 to 20% more in comparison to normal wood), whose microfibrillar angle is oriented parallel to the fiber axis (in contrast with the helical provision in the secondary layers) (Patten *et al.* 2007).

The presence of tension wood can cause problems in wood processing and use. During the drying process, defects such as deformation, torsion, bending, and cracking are developed and are associated, in particular, with the contraction characteristics, which differ when compared to normal wood (Sultana and Rahman 2013). Identifying, understanding, and analyzing the characteristics of rubber tree wood and the mechanisms involved in the tension wood formation, can provide subsidies for the plantations planning, aiming the best use of the wood at the end of the latex extraction period.

The purpose of this study was to characterize the anatomical structure of the wood panel clone of *Hevea brasiliensis* (Willd. ex Adr Jussieu) Muell. Arg. (PB 311), under the influence of two different canopy grafts. The study was conducted on the basis of quantitative anatomical characters present in the panel clone wood, whose objectives were: (1) to characterize the radial variation (direction pith-cambium); (2) to analyze the variation in tension, for opposite and normal wood; and (3) to analyze the canopy graft influence on the rubber tree panel clone anatomy.

EXPERIMENTAL

Materials

The clones of *Hevea brasiliensis* used in this study were granted by the company Plantações Michelin da Bahia LTDA, in experimental plantations located in Igrapiúna (Bahia/Brazil (13°48'51"S, 39°8'54"W), with spacing 8 x 2.5m.

Selection and wood samples collection of the rubber tree clones

Rubber trees were selected in a double-grafted system with variation in the canopy graft, aged 24 and 21 years, respectively: (1) root from seeds + clone PB 311 (panel) + clone FX 2784 (canopy); and (2) root from seeds + clone PB 311 (panel) + MDF 180 clone (canopy). The base and second grafts were performed at 8 to 9 months and 1.5 to 2 years of age (approximately 2.20m in height), respectively. Information on parental clones and country of origin of the respective clones are presented in table 1. For each situation, three trees were sampled. All the trees were stimulated with ethephon 4% (Ethrel ® 720, Bayer Crop Science, Research Triangle Park, NC, USA) aiming at the latex production. The choice of this double-grafted system was justified by the uneven diameter growth visual analysis among the clones of MDF 180 canopy and PB 311 panel (Fig. 1).

Table 1. Parental Clones and Country of Origin of the *Hevea brasiliensis* Clones Selected for the Present Study

Clone	Parental Clone	Country of Origin
FX 2784	F 4542 X AVROS 363	Brazil
MDF 180	Primary clone	Peru
PB 311	RRIM 600 X PB 235	Malaysia

MDF = Madre de Dios Firestone; FX = Ford Crossing; AVROS = Algemene Vereniging Rubber planters Oostkust Sumatra; PB = Prang Besar; RRIM = Rubber Research Institute of Malaysia. Source: Mattos et al. (2003).

From each tree, discs of wood were cut from the affected branch immediately below the insertion of canopy clone at right angles to the axis (Fig. 2). Samples of this material were deposited in the wood collection of the Forests Institute of the Federal University of Rio de Janeiro, with the following record numbers: 7714, 7715, 7716, 7717, 7718, and 7719. From the observation of the eccentric pith presence, specimens were observed in three radial regions covering the tension, of opposite and normal woods.

Fig. 1. Double-grafted trees of *Hevea brasiliensis* selected for this study. **a**: root from seed + PB 311 clone (panel) + FX 2784 clone (canopy). **b**: root from seed + PB 311 clone (panel) + MDF 180 clone (canopy). Plantações Michelin da Bahia LTDA, in plantations located in Igrapiúna, BA.

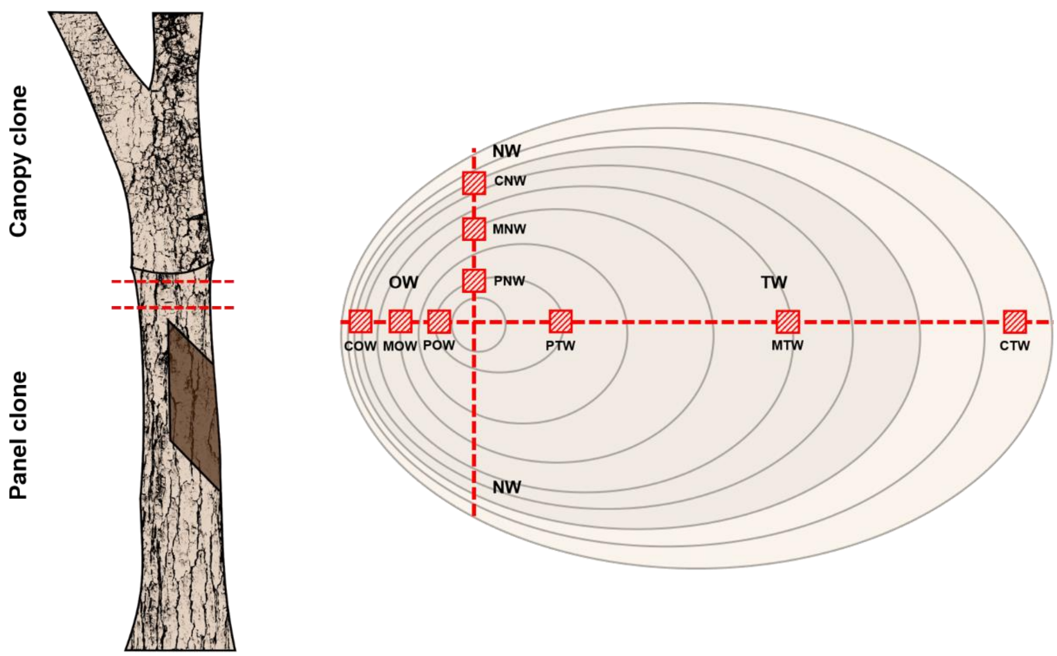

Fig. 2. Schematic drawing of obtaining wood discs and cross-sectional diagram of the trunk illustrating regions sampled for anatomical analysis. TW, OW, and NW = tension, for opposite and normal wood, respectively; PTW, POW, and PNW = pith of tension, for opposite and normal wood, respectively; MTW, MOW, and MNW = middle section of tension, for opposite and normal wood, respectively; CTW, COW, and CNW = cambium region of tension, for opposite and normal wood, respectively

Methods

Wood anatomy

For the histological cuts, transverse and longitudinal sections (radial and tangential) of 18 µm thickness were obtained in a microtome slide (MICRON HM 450) from the specimens. Then, the transverse sections were subjected to double staining with astra blue and safranin, in the proportion 9.5:0.5 (Bukatsch 1972). The lignified structures interact with safranin, acquiring red coloration, while the cellulosic structures react with the astra blue and, therefore, acquire blue coloration. This staining process allowed for the identification of the gelatinous fibers, rich in cellulose, often present in the tension wood. The longitudinal sections were subjected to safranin staining at 1% (Johansen 1940). Such cuts were used for the manufacturing of semi-permanent slides (Purvis *et al.* 1964). To assemble the permanent slides, the cuts after staining, were dehydrated in an alcohol series (20, 40, 60, 80, and 100%), treated with ethyl acetate, and set in resin.

For each specimen, wood fragments were selected in the direction of the fibers, for the tissue dissociation. The dissociation was performed according to methodology described by Franklin (1945), with changes in the temperature (70 ºC) and dissociation time (8 h). The fragments were stained with safranin at 1% and used for the semi-permanent slide manufacturing.

The histological cuts and dissociated tissues were employed in a quantitative microscopic study of the following anatomical characters: tangential diameter (µm), frequency (vessels/mm²) and length (µm) of the vessel elements; height (µm), width (µm), and frequency (radius/mm linear) of the rays; length (µm), total diameter (µm), and fiber wall thickness (µm).

All measurements were performed according to the standards established by the International Association of Wood Anatomy Committee (IAWA 1989). The image capturing was performed by means of a high-resolution camera coupled with an optical microscope *Olympus CX40* connected to TSView software 6.2.4.5 (Tucsen Imaging Technology Co., Limited, Fujian, China). The images were analyzed in the *software Image-Pro Plus*® 4.5.0.29.

Proportion of gelatinous fibers

For the determination of the proportion of gelatinous fibers, cross-sectional images containing such elements were analyzed in the Image-Pro Plus software using the "count/size" command (Kataria *et al.* 2012). The proportion was obtained by subtracting the blue component of the images (gelatinous fibers) from the red components (other lignified structures), manually demarcated (Purba *et al.* 2015).

Statistical analysis

After verifying the absence of normality in the residues (Shapiro-Wilk test, at the level of 95% confidence), the nonparametric Kruskal-Wallis test was performed, followed by the Bonferroni method for multiple comparisons among the types of woods and radial positions studied, both at 5% of significance. These analyses were performed using the statistical package *Action Stat* 3.2.60.1118. Multivariate associations were assessed among the anatomical characteristics of both double-grafted rubber trees, by a two-step cluster analysis. The grouping was performed according to the Bayesian Information Criterion (BIC), using for distance measured the log-likelihood. The significance of the variables within each cluster was determined by means of the Bonferroni test T-Test, at the level of 95% confidence. To do this, the statistical package IBM® SPSS® 20.0 was used.

RESULTS AND DISCUSSION

Wood Anatomy Features

No qualitative differences in the anatomy of the wood panel clone PB 311 was observed in the evaluated double-grafting systems, so the description below is valid for both cases.

Growth ring boundaries: These were slightly distinct and were possibly demarcated by fibrous areas.
Vessels: These showed that the wood was diffuse-porous. These were solitary vessels and were in radial multiples of 2 to 6, occasionally forming clusters, circular to oval section. There were simple perforation plates and the presence of appendages, with varying sizes, often at both ends. The inter-vessel pits were alternate; the vessel-ray pits had very reduced borders to apparently simple ones; the pits were rounded or angular. The tangential diameter was 71.24 µm to 371.39 µm. The vessel frequency (vessels/mm²) was 1 to 31. The vessel element length was from 536.52 µm to 1060.91 µm. There was a presence of common (Fig. 3A and 4B/D) and occasionally sclerotic tyloses in the region near the pith (Fig. 3E and 4C/E).
Fibers: These were non-septate, with thin-to-thick-walls, and with a length of 1102.40 µm to 1924.91 µm and simple pits. There was a presence of gelatinous fibers (Fig. 3A/B).
Axial parenchyma: This showed banded parenchyma reticulate.
Rays: The rays were numerous, ranging from 4 to 14 rays / linear mm. They were multi-serious with a width of 1 to 5 cells (Fig. 3C/D/E), They showed a heterogeneous cellular composition with procumbent body ray cells with mostly 2 to 4 rows of upright and/or square marginal cells (Fig. 3F). There was a presence of aggregate rays.
Mineral inclusions: There were calcium oxalate prismatic crystals present in the upright and/or square ray cells, in the axial parenchyma cells (occasionally forming short chains), and in the tyloses (Fig. 3D and 4D/E).

Both the double-grafted rubber trees presented vessels obstructed by common tyloses and occasionally sclerified in the region close to the pith. The obstruction can occur in a natural way, with the sapwood formation, or in response to biotic and abiotic stresses (attacks of pathogens, mechanical injuries, drought, or frost) (Feng *et al.* 2013; Dufraisse *et al.* 2017; Lesniewska *et al.* 2017; Pérez-de-Lis *et al.* 2018). The tyloses location in the material studied indicates that the formation happened naturally with the xylem aging. A wide variety of organic and mineral components may be contained in the tyloses formation process, among which stand out: gums, resins, starch, crystals, and phenolic compounds (De Micco *et al.* 2016). In this study, the presence of prismatic crystals of calcium oxalate associated with tyloses was identified.

Proportion of Gelatinous Fibers

The presence of gelatinous fibers in all the wood for both the studied double-grafted rubber trees was observed (Fig. 5). Among the double-grafted trees, the PB 311 clone under the influence of the FX 2784 canopy graft presented higher proportions of these elements in all radial positions, except in the region near the pith of the normal wood. The double-grafted PB 311 + FX 2784 showed a higher proportion of fibers in the normal and opposite wood, which were concentrated in the middle and cambium regions, respectively.

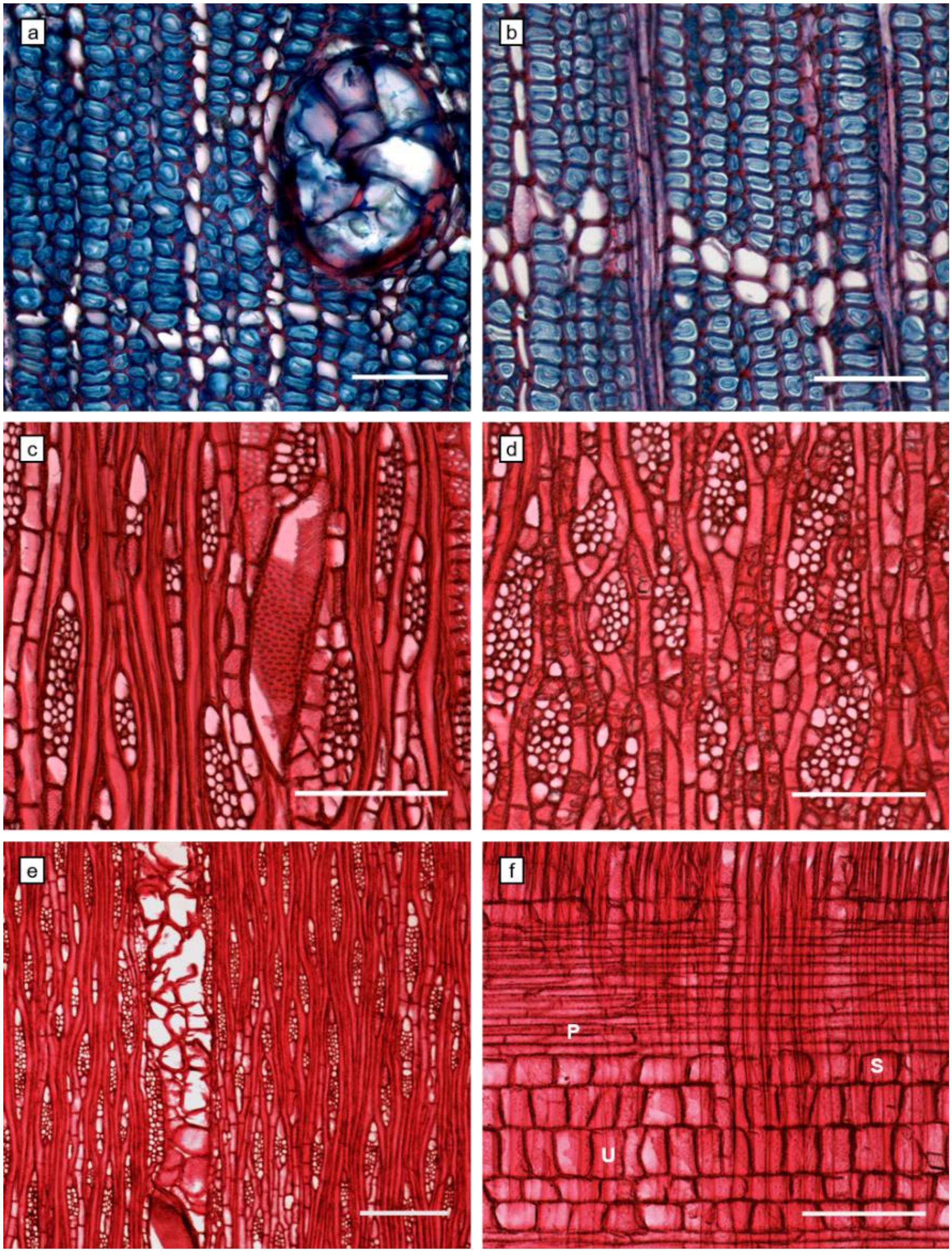

Fig. 3. Wood anatomy of *Hevea brasiliensis*; a through b: cross sections; c through e: tangential sections; f: radial sections. a: common tyloses and abundance of gelatinous fibers; b: presence of double-layer gelatinous fibers; c-e: ray width 1 to 5 cells, highlighted vessels with simple perforation plates (c), abundance of prismatic crystals in axial parenchyma cells (d) and presence of sclerotic tyloses (e); f: heterogeneous ray cellular composition: procumbent (P) body ray cells, with mostly 2-4 rows of upright (U) and/or square (S) marginal cells. Scale bar: a/b = 100 µm; c/d/f = 200 µm; e = 300 µm

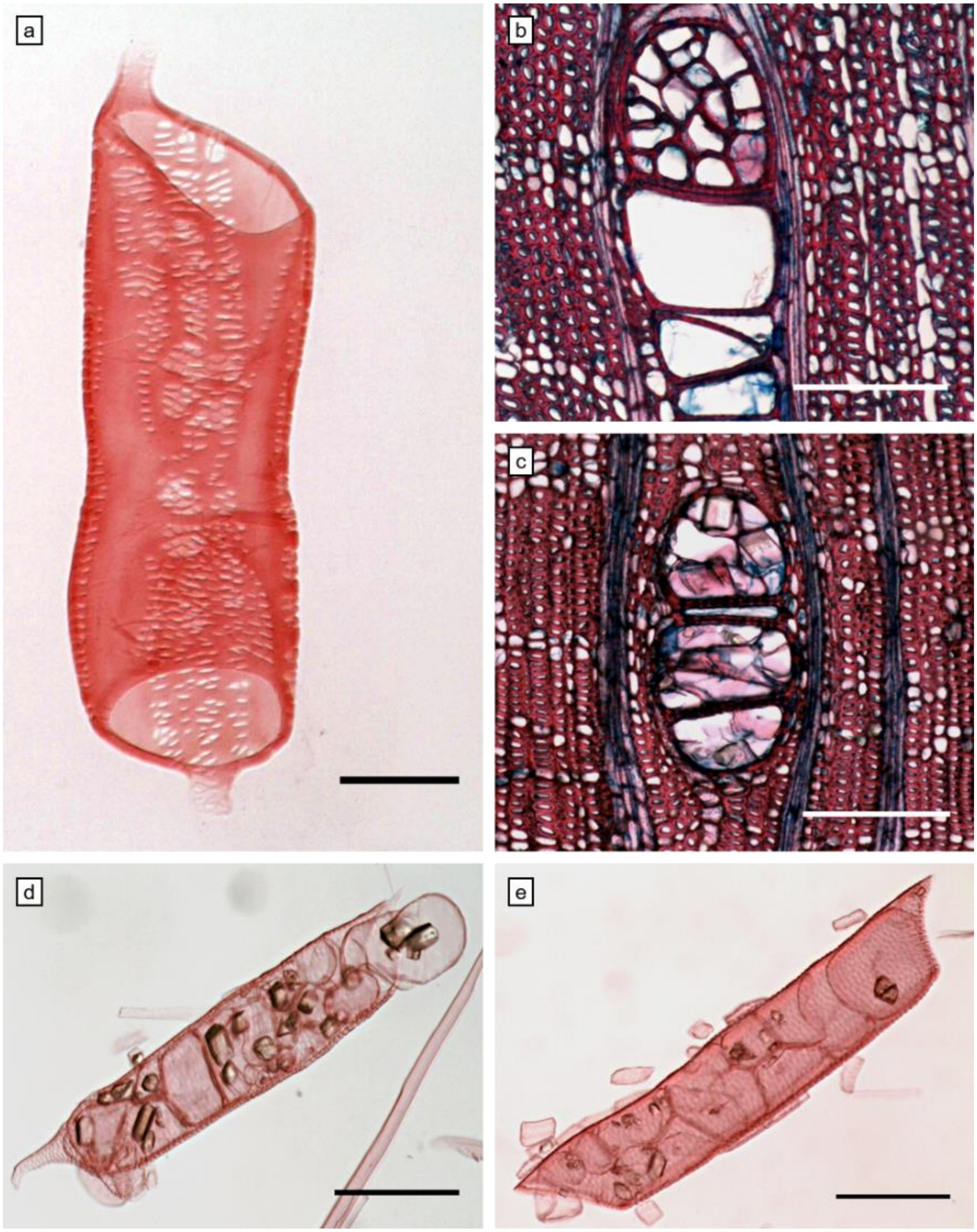

Fig. 4. Wood anatomy and cell types of *Hevea brasiliensis*. a/d/e: maceration; b/c: cross-section. a: vessel element, highlighted vessels with simple perforation plates, presence of appendages at both ends and vessel-ray pits with much reduced borders to apparently simple: rounded or angular pits; b/d: sclerotic tyloses with prismatic crystals (d); c/e: common tyloses with prismatic crystals (e). Scale bar: a: 100 µm; b-e: 200 µm

The PB 311 + MDF 180 presented a different pattern, with larger proportions in the regions near the pith and cambium in opposite and normal wood, respectively. In both double-grafted trees, it is possible to observe that tension wood concentrates the smallest proportions of these fibers, especially in the cambium.

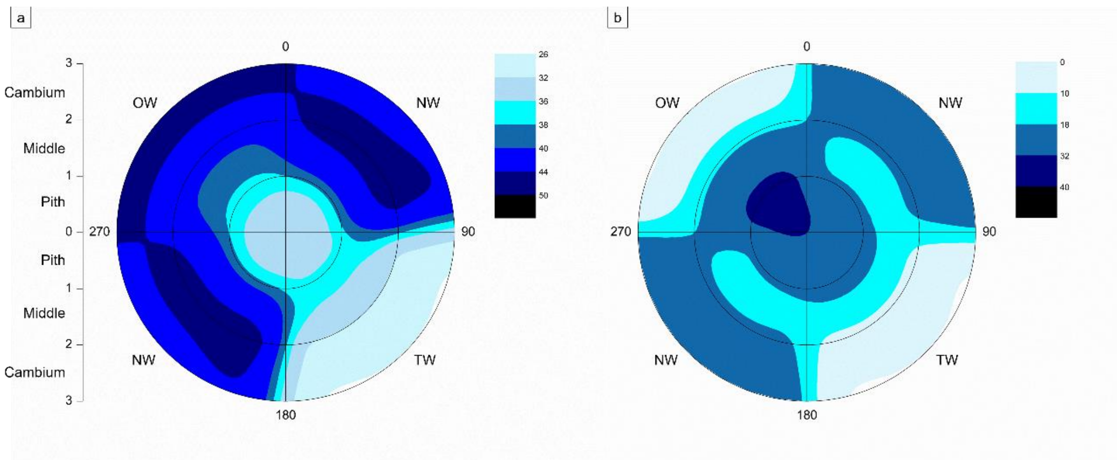

Fig. 5. Proportion of gelatinous fibers in tension, in the opposite and normal wood of PB 311 clone, under the influence of FX 2784 (a) and MDF 180 (b) canopy grafts, of *Hevea brasiliensis*. The gradual increase in the shades of blue indicates a higher proportion of gelatinous fibers in the portion of analyzed wood. The OW, TW, and NW = opposite, tension, and normal wood, respectively.

The occurrence of tension wood already has been observed in many arboreal species, among them *Acacia* sp., *Eucalyptus* sp., *Populus* sp., *Fagus sylvatica*, *Sarcandra glabra*, *Salix* spp., *Liriodendron tulipifera*, and *Hevea brasiliensis* (Hillis *et al.* 2004; Monteiro *et al.* 2010; Jin *et al.* 2011; Li *et al.* 2013; Brereton *et al.* 2015; Gritsch *et al.* 2015; Nawawi *et al.* 2016; Ramos *et al.* 2016). In response to environmental factors and gravitational stimuli, anatomical changes occur, resulting from the tension wood formation, aiming at the stem reorientation under these conditions. The reaction wood, in hardwoods, generates a tension force that assists in the re-establishing of vertical growth of the wood, on the upper side of the stem inclination (Clair *et al.* 2006; Ruelle *et al.* 2006). The tension generated is attributed to the cellulose microfibril contraction present in the gelatinous layer (G layer) of the fibers typically associated to this wood (gelatinous fibers) (Clair *et al.* 2011). Due to the hydrophilic nature of the G layer and the frequent presence in xeric environments, the gelatinous fibers have also been associated with water storage (Marcati *et al.* 2001). In addition, recent studies indicate that these can also serve as aluminum storage, avoiding the toxicity of this element to plants (Milanez *et al.* 2017). In spite of the presence of reaction woods in the rubber trees of the present study, through the pith eccentricity, the gelatinous fibers were not associated exclusively with the tension wood, being observed in both opposite and normal wood. This indicates that the *H. brasiliensis* presents a pattern of differentiated response regarding the formation of this wood. Similar results were observed by Ramos *et al.* (2016) in rubber trees with 53 years of age, which showed a greater proportion of gelatinous fibers in the opposite wood.

Radial Variation and Anatomy of Tension, Opposite, and Normal Wood

The quantitative anatomical variables regarding the vessel elements, rays, and non-gelatinous fibers, to the PB 311 clone under the canopy FX 2784 and MDF 180 graft influence, are described in Table 2.

Table 2. Descriptive Statistics (Median Followed by Interquartile Deviation, in Parentheses) and Multiple Comparisons of Quantitative Anatomical Elements in PB 311 Clone (FX 2784 and MDF 180 Canopy Grafts) of *Hevea brasiliensis*

Canopy Graft	Wood	Vessels Elements								
		Tangential Diameter (µm)			Vessels/mm²			Length (µm)		
		P	M	C	P	M	C	P	M	C
FX 2784	OW	142.02^{cC} (34.96)	174.68^{bA} (66.60)	205.60^{aA} (56.99)	6^{aA} (3)	5^{bA} (3)	4^{bA} (3)	755.73^{cB} (337.01)	876.70^{bA} (230.12)	1000.11^{aA} (229.15)
	TW	171.84^{cB} (31.76)	191.35^{bA} (51.26)	205.34^{aA} (43.52)	5^{aB} (4)	4^{bB} (4)	4^{bA} (3)	785.51^{cB} (232.54)	887.69^{bA} (344.17)	964.93^{aA} (275.36)
	NW	180.49^{aA} (35.02)	192.80^{aA} (44.84)	186.12^{aB} (38.01)	4^{aB} (3)	4^{aB} (2)	3^{aA} (4)	889.56^{bA} (189.38)	959.32^{abA} (243.06)	985.08^{aA} (272.52)
MDF 180	OW	152.69^{bB} (49.44)	190.89^{aA} (43.25)	154.87^{bA} (64.90)	5^{bA} (3)	5^{bA} (3)	6^{aA} (6)	848.11^{abA} (243.06)	870.75^{aA} (263.06)	738.46^{bA} (241.28)
	TW	157.06^{bB} (42.44)	175.76^{aB} (48.08)	150.94^{bA} (66.57)	4^{aB} (2)	3^{aB} (3)	4^{aB} (4)	819.41^{aA} (229.79)	790.33^{aB} (178.05)	697.94^{bB} (218.14)
	NW	180.63^{bA} (49.79)	191.30^{aA} (49.28)	127.53^{cB} (54.93)	4^{bB} (3)	4^{bAB} (4)	6^{aA} (3)	878.58^{aA} (257.97)	875.14^{aA} (269.76)	824.13^{aA} (283.61)
Rays										
		Height (µm)			Width (µm)			Ray/ Linear mm		
FX 2784	OW	344.84^{cB} (126.35)	385.22^{bB} (146.86)	450.50^{aA} (139.91)	29.93^{bC} (7.30)	36.43^{aA} (11.30)	37.83^{aB} (8.90)	8^{aB} (3)	8^{aA} (2)	8^{aA} (2)
	TW	408.72^{bA} (140.39)	442.58^{aA} (140.33)	484.63^{aA} (146.50)	33.83^{cB} (7.80)	36.46^{bA} (11.01)	44.24^{aA} (9.70)	9^{aA} (2)	9^{aA} (2)	8^{aA} (1)
	NW	433.33^{bA} (149.44)	419.48^{bB} (125.56)	477.75^{aA} (185.21)	36.46^{bA} (9.04)	35.13^{bA} (8.51)	39.06^{aB} (10.36)	8^{aB} (3)	8^{aA} (2)	8^{aA} (2)
MDF 180	OW	386.78^{aB} (148.18)	351.34^{aA} (93.36)	368.25^{aA} (136.35)	36.46^{bAB} (9.15)	37.74^{bA} (8.96)	48.15^{aA} (21.62)	7^{aB} (4)	7^{aC} (2)	8^{aB} (2)
	TW	421.67^{aA} (118.33)	400.99^{aA} (133.99)	365.68^{bAB} (117.86)	37.76^{bA} (10.96)	37.76^{bA} (7.91)	44.24^{aA} (15.04)	8^{bA} (3)	9^{aA} (2)	9^{abA} (3)
	NW	360.56^{aC} (89.23)	365.27^{aA} (81.33)	327.27^{bB} (120.14)	33.93^{cB} (6.58)	37.74^{bA} (6.48)	44.41^{aA} (10.85)	7^{bB} (3)	8^{aB} (2)	8^{aAB} (2)
Non-Gelatinous Fibers										
		Length (µm)			Total Diameter (µm)			Wall Thickness (µm)		
FX 2784	OW	1359.81^{cB} (360.07)	1530.21^{bA} (304.25)	1718.08^{aA} (325.32)	26.91^{aAB} (7.43)	27.88^{aA} (5.89)	28.31^{aB} (7.55)	3.89^{bB} (0.96)	4.54^{aA} (1.89)	4.75^{aA} (1.22)
	TW	1268.79^{cB} (214.10)	1510.73^{bA} (307.88)	1715.54^{aA} (304.65)	25.92^{bB} (5.30)	28.96^{aA} (6.82)	30.19^{aA} (6.91)	3.43^{cC} (1.08)	4.29^{bA} (1.71)	4.63^{aA} (1.57)
	NW	1496.46^{bA} (165.39)	1471.23^{bA} (317.13)	1691.55^{aA} (344.51)	28.41^{bA} (5.31)	28.98^{bA} (8.34)	31.15^{aA} (5.38)	4.52^{bA} (1.38)	4.65^{abA} (1.40)	4.74^{aA} (1.27)

MDF 180	**OW**	1346.05^{cC} (186.05)	1595.34^{aA} (272.55)	1472.33^{bA} (392.71)	27.30^{bA} (5.31)	31.31^{aA} (6.39)	28.09^{bA} (5.91)	3.67^{bC} (0.90)	5.46^{aA} (1.43)	5.42^{aA} (0.92)
	TW	1426.98^{aB} (214.26)	1460.23^{aB} (319.51)	1264.11^{bB} (254.11)	26.82^{aA} (5.68)	28.26^{aB} (8.13)	23.81^{bC} (6.46)	4.13^{bB} (1.39)	4.63^{aB} (2.23)	4.55^{aB} (0.86)
	NW	1506.86^{bA} (235.05)	1653.18^{aA} (247.49)	1458.54^{bA} (402.66)	27.99^{aA} (4.40)	29.05^{aB} (6.46)	26.41^{bB} (4.52)	4.80^{abA} (0.99)	5.14^{aA} (1.22)	4.63^{bB} (1.45)

P = Pith; M = Middle section; C = Cambium region; OW, TW, and NW = Opposite, Tension, and Normal wood, respectively
Equal lower and uppercase letters indicate no significant difference between radial sections and woods, respectively, by Bonferroni method ($p > 0.05$).
The occurrence of ambiguities (ab/AB) was interpreted as a non-significant difference between the analyzed variables.

In general, the pattern of maturation in woody plants, which is associated with the gradual increase in the number of xylem cells, becomes evident as the pith separation occurs (Chowdhury *et al.* 2009; Kojima *et al.* 2009; Nugroho *et al.* 2012). This behavior is attributed mainly to the auxin concentrations (Lovisolo *et al.* 2002), which are higher in the regions of synthesis for the hormones of this plant (shoot apical meristem, young leaves, developing fruits and seeds) (Aloni 2001; Sorce *et al.* 2013). The caulinar apex is the main source of auxin to woody tissues, which is transported polarly to the vascular cambium and distributed radially to the xylem and phloem *via* efflux carriers located on the sides of the cambium cells and their derivatives (Sundberg and Uggla 1998; Petrásek and Friml 2009). Thus, a concentration gradient of this plant hormone along the radial section, where elevated, intermediate, and low levels are observed in the cambium and in the elongation and maturation areas, respectively (Bhalerao and Bennett 2003; Bhalerao and Fischer 2014; Immanen *et al.* 2016). Therefore, it is suggested that the cell division and expansion processes are signaled by high and middle concentrations of auxin, while the deposition of the secondary cell wall is induced by low concentrations (Ljung 2013).

In addition to the intrinsic species characteristics (genetic material, ontogeny), other factors can influence the wood anatomy, such as: silvicultural traits and climate conditions (temperature, water, and nutrient availability) (Naji *et al.* 2013a; b; Novak *et al.* 2013; Fortunel *et al.* 2014; Ziaco *et al.* 2014; Pfautsch *et al.* 2016). The initial planting density, for example, can influence the tree growth, affecting the production and quality of the wood generated and causing anatomical changes because of the degree of densification (Naji *et al.* 2014). The anatomic characters can be modulated, still, depending on the rainfall regime and abiotic stress (flooding and drought, for example), demonstrating the adaptation capacity in adverse conditions for the tree species (Fichtler and Worbes 2012).

Vessels Elements

Double-grafted rubber tree PB 311 + FX 2784

In the opposite and tension woods, the anatomical characters corresponding to the vessel elements varied in function in the radial position (pith, middle, and cambium). In both woods, tangential diameter and the vessel element length increased in the pith-cambium direction, while the frequency (vessels/mm²) reduced. For the normal wood, no significant differences were observed for all the analyzed variables.

Regarding the variation among the woods, in the region close to the pith, the normal wood showed vessel elements with larger diameters and length and lower frequency, followed by tension and opposite woods. The middle region showed no significant variation, with the exception of the number of vessels/mm², which was greater in the opposite wood. Near the cambium, only the tangential diameter differed among the woods, being lower in the normal wood and equal in the other woods.

The vessels play a fundamental role in the woody angiosperm organism, promoting the rise of water and minerals from the roots to the leaves (Tyree and Ewers 1991; Pratt *et al.* 2008; Santini *et al.* 2016; Zhu *et al.* 2017). The longitudinal variation of these cellular elements was determined to ensure the hydraulic efficiency, promoting increases in diameters from the top to the bottom (Aloni and Zimmermann 1983; Olson *et al.* 2014; Petit and Crivellaro 2014). In general, with all the characteristics related to the vessel elements (tangential diameter, length, and frequency), the double-grafted PB 311+FX 2784 presented a response pattern in accordance to those described in the literature, that is, smaller and narrower vessel elements next to the pith and, consequently, more frequent. The formation of narrow vessels in the plant's accelerated growth period may be considered strategy to ensure greater safety in the transport of water, reducing the vulnerability to embolism (Zhao 2015). Such variation in the vessel diameters may be explained by changes in the auxin concentrations (Aloni 2015). In the plant's juvenile phase, the synthesis points of this plant hormone exert greater influence on the cells, since, under these conditions, the proportion of young leaves is high. High levels of auxin promote rapid cell differentiation, causing accelerated secondary wall deposition, and limiting the cell size. When the levels are reduced, the differentiation is slow, that is, there is more time for the cell's development until the secondary wall formation occurs (Aloni and Zimmermann 1983).

Double-grafted rubber tree PB 311 + MDF 180

In the three woods, the vessel tangential diameter was larger in the middle region, reducing near the cambium. In the opposite wood, the vessel element length did not differ statistically, while the number of vessels/mm² increased toward the pith-cambium. The frequency of vessel elements did not differ in the three radial positions for the tension wood, while the length of these elements was smaller next to the cambium. In the normal wood, the vessel elements showed increased frequency only in the region near the cambium, keeping constant in other regions. A significant difference was not observed for the vessel element length, in this wood.

Next to the pith, the vessel element tangential diameter and frequency were inversely proportional in the opposite and normal woods, not different in the length of these elements. In the middle region, the tension wood presented the lowest values for all variables, keeping low vessel frequencies even when they presented lower diameters. In the cambium, the tangential diameter was lower for the normal wood; whereas the vessel element frequency and length were smaller in the tension wood.

This double-grafted tree presented a distinct pattern to those described in the literature, with an increase in the tangential diameter of the vessels in the middle portion of the wood. The reduction of these variables in the region close to the cambium was reflected in the frequency of these elements, which increased significantly. Because of the increase in demands for mechanical stability and efficiency in conducting water and nutrients throughout the tree's development, the cell wall size and thickening could be modulated in the function of plant needs (Rungwattana and Hietz 2017). The results obtained for this double-grafted tree suggest a possible response sensitivity of the panel clone vessels to change in the diameter growth of the canopy clone, demarcating the moment in which the clone's development became uneven.

Rays

Double-grafted rubber tree PB 311 + FX 2784

In the three woods, the characteristics concerning the height and width of the rays increased towards the pith-cambium, while the frequency (rays/mm linear) showed no significant difference among the radial positions.

In the region close to the pith, the normal wood presented larger and wider rays, when compared to the same position in the other woods. Only the amount of rays/mm linear exhibited distinct behavior in this region, being higher for the tension wood. In the middle region, only the variable "height of rays" differed statistically among the woods, being greater for the tension wood. Next to the cambium, a distinction among the woods was observed in the width of the rays, a superior characteristic to the tension wood.

The rays are composed of parenchyma cells whose main function is to store nonstructural carbohydrates (starch, for example) and radially lead water and photoassimilates (Pfautsch *et al.* 2015; Plavcová and Jansen 2015; Bowen and Pate 2017). In addition, they have other functions: the protection against herbivory, by means of the deposition of tannins, terpenoids, crystals, and silica (Hudgins *et al.* 2003; Hara *et al.* 2006; Keeling and Bohlmann 2006); help in the cambium regeneration after damage caused by fire, drought, or frost (Carlquist 2012; Morris *et al.* 2016); and embolism reversal by means of ions and assimilates transfer to the vessels (Brodersen *et al.* 2010). Species such as the rubber tree, whose composition is heterogeneous, feature procumbent cells in the central portion of these elements and upright cells on the ends. The differentiated guidance of these cells is related to the conduction direction, *i.e.*, procumbent cells conduct radially while the upright cells are responsible for vertical conduction (Sokolowska and Zagórska-Marek 2012; Spicer 2014). Increases in the ray height and width of the double-grafted PB 311+FX 2784 toward the pith-cambium was observed. This was due to the abundance of procumbent cells because of the need for a greater volume of photoassimilates conducted horizontally as there is in the tree's growth (Carlquist 2012).

Double-grafted rubber tree PB 311 + MDF 180

Toward the pith-cambium direction, the ray width increased in the three woods. In the opposite wood, the rays showed equal height and frequency in all regions. The height and width of the rays were inversely proportional both in the tension wood as in the normal wood, featuring smaller and wider rays in the region close to the cambium. An increase was observed in the amount of rays/mm linear in the pith-cambium direction to the normal wood, while for the tension wood there was no difference among the positions. The rays next to the cambium reduced in height, which, in theory, could affect the radial storage and the solutes transport in this region. In contrast, there was an average increase in width of 17.2% in relation to the middle portion of the wood, indicating a possible compensation.

In the regions close to the pith and the middle, the tension wood presented longer and numerous rays, when compared to the other woods, as well as a constant width. In the cambium, the characters did not differ statistically among the woods.

Non-Gelatinous Fibers

Double-grafted rubber tree PB 311 + FX 2784

The length of the non-gelatinous fibers increased toward the pith-cambium, for the three analyzed woods. In the opposite wood, the total diameter of the fibers did not differ statistically among the radial positions, showing increases only in wall thickness as they approached the cambium. For the tension wood, both the total diameter and the wall thickness of the fibers increased from the pith to the cambium. The normal wood fibers presented an increase in the total diameter while the wall thickness remained statistically equal.

In the region close to the pith, the normal wood presented fibers higher in length, diameter, and wall thickness. The tension and opposite woods differed only in the wall thickness in this region, with the first wood featuring thicker fibers. In the middle position, the variables did not differ in the three woods. Next to the cambium, a variation among the woods was observed only for the total fibers diameter, which was lower in the opposite wood.

The fibers differentiation process along the stem is assigned to the gibberellin in synergism with auxin. The gibberellin precursor (GA_{20}) is produced in mature leaves and transported to the cambium *via* phloem where it is converted into plant hormone in its bioactive form (GA_1), activating the cambium and inducing the fiber differentiation (Xiao *et al.* 2010; Dayan *et al.* 2012). The bioactive forms are concentrated mainly in the expansion zone of the xylem cells in differentiation, *i.e.*, the operation in cell elongation, producing longer fibers, consequently (Dayan *et al.* 2012; Withanage *et al.* 2015; Xiao *et al.* 2016). The fiber characteristics observed in the double-grafted PB 311 +FX 2784 increased toward the pith-cambium. The variation in the fiber wall length, diameter, and thickness is related to the tree age (juvenile and adult woods). The fibers present in the juvenile wood were smaller, thinner, and with thin-walled, gradually increasing along the transition zone to stabilize in the adult wood (Ferreira *et al.* 2011; Ramos *et al.* 2011; Trevisan *et al.* 2017). For the rubber trees, studies have demonstrated that between 40 to 55 mm away from the pith the mature wood formation occurs (Ferreira *et al.* 2011; Severo *et al.* 2013).

Double-grafted rubber tree PB 311 + MDF 180

In the opposite wood, the non-gelatinous fibers showed greater length and diameter in the middle portion of the radial section, increasing the thickness as the pith separation occurred. In the tension wood, the fibers reduced the length and diameter in the cambium, thickening the wall in a similar way to the opposite wood. The normal wood's fiber length, like the opposite wood, was higher in the middle region, reducing the cambium diameter without, however, changing the wall thickness. The reduction of the fiber length and total diameter in the region close to the cambium indicated early maturation of these trees, that is, it was assumed that this reduction was due to the oscillations commonly associated with adult wood.

Among the woods, in the region near the pith, the normal wood presented larger and thicker fibers, followed by the tension and opposite woods, respectively. The middle region concentrated the highest values among all the characters evaluated for each type of wood. In this region, both the opposite and the normal wood had long fibers with thick walls, but only the first differed statistically as to the diameter. In the portion next to the cambium, the tension wood fibers were smaller in both length and diameter, compared to the other woods; the wall thickness was larger in the opposite wood.

The results obtained in this study show that the quantitative anatomical characters vary in the function of the different types of wood (tension, opposite, and normal) in each radial position, at random. The anatomical adjustments observed in the same clone highlights the dynamic process, which is the tension wood formation, in virtue of the range of factors capable of modulating the wood anatomy in field conditions. In the present study, the anatomical variations among the woods were frequently associated with the region next to the pith, with the exception of the total diameter of the fiber of the double-grafted PB 311+FX 2784 and the length of the vessel elements, ray width, and the total diameter of the fiber of the double-grafted PB 311+MDF 180, which did not differ statistically. During the first few years of development, the trees are more prone to the tension wood formation, since they are more fragile and flexible during this stage. Studies conducted in young trees showed the response rapidity at this stage of development (Jourez *et al.* 2001a; b; Ruelle *et al.* 2007; Nugroho *et al.* 2013).

Influence of Canopy Graft on the Quantitative Anatomical Characteristics of Panel Clone

The two-step cluster analysis distinguished both of the double-grafted trees analyzed on the basis of quantitative anatomical characters, indicating a possible influence of the canopy graft in the panel clone anatomy. The formed groups were composed by 100% of the data regarding each studied material, reinforcing the robustness of this analysis in composing the clusters (Fig. 6).

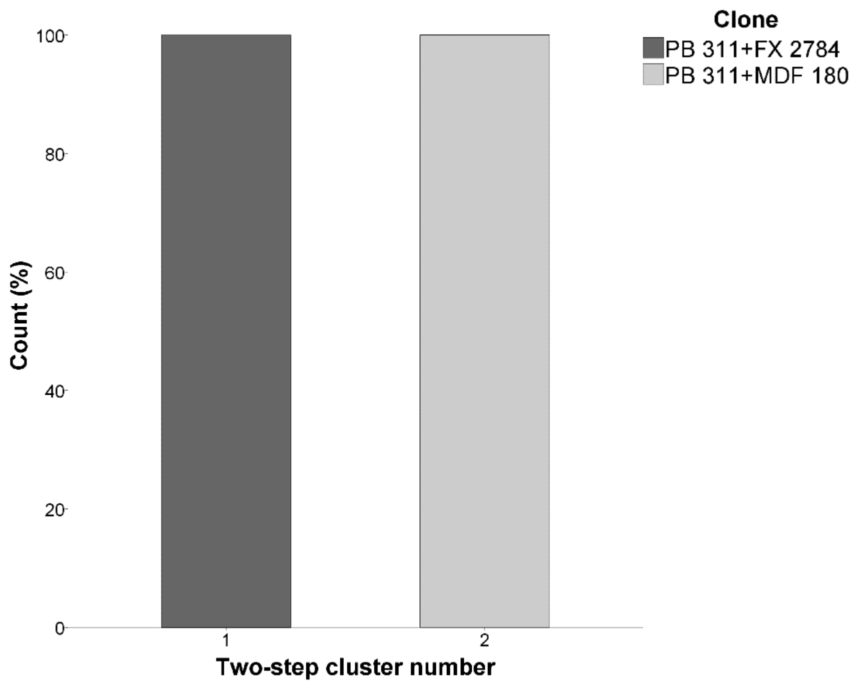

Fig. 6. The composition and size of each cluster

In both clusters, the variable "ray height" was the most important in the tree segmentation, followed by the "vessel tangential diameter" (Table 3). Comparing the groups formed, the cluster 1, composed by the double-grafted PB 311 + FX 2784, presented longer vessel elements, with larger diameters and lower frequency; more numerous rays, tall and thin; and non-gelatinous long fibers, with greater total diameter and less thickness.

Table 3. Importance of Anatomical Characters for Clusters Segmentation

Anatomical Characters	Cluster	
	1 (PB 311+FX 2784)	2 (PB 311+MDF 180)
* Rays Height (µm)	444.79 (428.18)	378.91 (368.58)
* Tangential Diameter of Vessels (µm)	185.66 (182.73)	167.23 (167.70)
* Vessel Element Length (µm)	892.40 (899.32)	814.84 (812.47)
* Rays Width (µm)	36.84 (36.46)	40.23 (38.53)
* Rays/ Linear mm	8.47 (8)	7.98 (8)
* Fibers Wall Thickness (µm)	4.44 (4.35)	4.72 (4.69)
* Fibers Length (µm)	1531.52 (1515.69)	1480.25 (1467.99)
* Total Diameter of Fibers (µm)	28.63 (28.47)	27.93 (27.74)
^{ni} Vessels/mm^2	4.67 (4)	4.88 (4)

Note: * important; ^{ni} not important. The mean is followed by median, in parentheses. Clustering was performed using the Bayesian Information Criterion (BIC), using as the distance measure the probability log. Variables significance within each cluster was determined by Bonferroni t-student test, at 95% confidence level.

As observed in the field, the canopy graft of MDF 180 presented diameter growth higher than the panel clone PB 311, possibly causing an imbalance between the canopy and the trunk of these trees. By the two-step cluster analysis, it was possible to observe a pattern in the anatomical structure of the two quantitative double-grafted elements studied, indicating that this possible canopy disproportion may have resulted in changes in the wood anatomy.

The variables "ray height" and "vessel elements tangential diameter" were the characteristics of major importance for the double-grafted segmentation. This suggests that the adaptation process of extra load, caused by the higher development of the canopy, can be related to the storage mechanisms, axial and radial conduction of carbohydrates, and the water and assimilates. The PB 311 clone under the influence of the MDF 180 canopy showed, on average, narrower vessels, indicating a possible strategy to ensure efficient and safe conduction to the aerial part, whose demand for water and nutrients tends to be larger. Despite the average ray height having been lower in this double-grafted tree, there was a possible compensation in width.

The lumen diameter and the fiber wall thickness are important characteristics regarding the preliminary analysis of the wood mechanical resistance. The results obtained for the double-grafted PB 311 + MDF 180 suggest that the changes in these cellular elements were in function of increased mechanical resistance. Fibers with thicker walls promote the wood structural adjustments contributing to the tree sustainability and, consequently, increasing the mechanical resistance (Longui *et al.* 2012; França *et al.* 2015). The lumen diameter, in turn, is related in reverse, that is, the smaller, the more the wood resistance tends to be (Evangelista *et al.* 2010).

CONCLUSIONS

1. The quantitative characteristics of the vessel elements, rays, and fibers of the double-grafted PB 311 + FX 2784 and PB 311 + MDF 180 showed a distinct pattern of radial variation between them.
2. The clone PB 311, under the influence of the MDF 180 canopy graft, presented variations in vessel elements, rays, and fibers in regions close to the cambium, indicating the ability of the same to modulate its anatomical structure in function of the extra loads (canopy uneven diameter growth).
3. The gelatinous fiber distribution pattern in the analyzed tree wood indicates that the rubber tree has a pattern of differentiated response regarding the tension wood formation.
4. The quantitative anatomical characters vary in function of the different types of wood (tension, opposite, and normal) in each radial position, at random.
5. The wood cells of both double-grafted rubber trees showed variations in their morphology. This indicates that the diametrical disproportion between panel and canopy causes modifications in wood anatomy. Thus, different canopy grafts result in changes in the anatomical structure of the panel graft.

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