Original Article

Bulletin of Pure and Applied Sciences.
Vol.36 B (Botany), Issue (No.1) 2017
Print version ISSN 0970 4612
Online version ISSN 2320 3196
DOI 10.5958/2320-3196.2017.00001.5

EVALUATIONS OF VESSEL ELEMENTS AND RAYS IN THE TRUNK OF *THEOBROMA CACAO*

J. E. Otoide

Author's Affiliation: J. E. Otoide, Department of Plant Science and Biotechnology Faculty of Science, Ekiti State University, Ado-Ekiti.

Corresponding Author: J. E. Otoide, Department of Plant Science and Biotechnology Faculty of Science, Ekiti State University, Ado-Ekiti.
Email: joeotoidejo@yahoo.com, jonathan.otoide@eksu.edu.ng

Received on 26.03.2017,
Accepted on 28.5.2017

Abstract

The morphology of the vessel elements and rays in the trunk of a fruiting tree of *Theobroma cacao* were carried out with the aid of light microscope. The vessel elements were short and narrow being less than 350µm and occurred in solitary and multiple forms. The ray tissues were both homogeneous and heterogeneous in distribution. Vessel and ray morphology such as length, diameter and height were evaluated, fifty vessels and rays were measured and thereafter the data were subjected to ANOVA and Duncan Multiple Range Test (D.M.R.T). The total average length (µm) and diameter of the vessels were 340.69 ± 182.27 and 78.62 ± 32.66 respectively. The pool means of vessel lengths (µm) at base, middle and top positions of the trunk were 422.31 ± 152.20, 349.23 ± 163.23 and 250.53 ± 189.39 respectively. The diameters (µm) of the vessels on the other hand, were averages of 86.34 ± 35.96, 79.24 ± 32.06 and 70.28 ± 27.96 for the base, middle and top positions of the trunk respectively. The total height (µm) and diameter (µm) of the rays were averages of 0.79 ± 0.47 and 74.85 ± 36.68 respectively. From the axial direction, the pool means of the height of rays were 1.09 ± 0.33, 0.78 ± 0.44 and 0.52 ± 0.45 for the base, middle and top regions of the trunk respectively. At $P \leq 0.05$, the length and diameter of vessels decreased significantly from the base to the top of the trunk. Similarly, the heights and diameters of rays in the trunk decreased significantly from base to top. The physiological advantages of the types of vessel and rays were discussed vis- a- vis the previous assertions of authors within the present context.

Keywords: vessels, rays, trunk, means, length and diameter, physiological advantage.

INTRODUCTION

Theobroma cacao Linn belongs to the family, Sterculiaceae (Burkill, 2000; Olorode, 2012). It is a small tree which grows up to 7m high or more under shade. The genus Theobroma has 22 species with *T. cacao* the only one cultivated. The fruits are the economically important part of the plant. They are borne cauliflorously on the older wood of the trunk and main branches and morphologically are indehiscent drupes (Burkill, 2000).

In Europe and America the beans are manufactured into the drink, cocoa and into chocolate. Cocoa-butter is one of the by-products in the manufacture. The young pods of cocoa are sometimes eaten as okro. As the seeds contain theobromine, cocoa is a stimulant to heart, kidneys and muscles and a diuretic (Ainslie). Theobromine has been proved to be poisonous to Cattle, Sheep, Pigs and Chickens (Irvine, 1961).

It is perceived that in the recent time our knowledge of the internal structure of the woods of tropical and temperate plants of economic and commercial values is poorly updated at a snail speed. The hardwoods in particular have not received the needed attention from the wood scientists. This scenario had made information about the cell and tissue structures in the plants scanty and not properly documented. This perception corroborates the earlier observation and demand made by Gill *et al.,* (1983), Gill and Ogunlowo (1988) and Metcalfe (1972) respectively. In the light of these, the author and his collaborators have in recent time, provided information on the type and structure of cells and tissues in the trunk of some commercial wood species in the tropics with a view to providing new and accessible relevant research information to complement the already existing few but old ones. The previous works of the present author reported in Otoide *et al.,* (2012), Otoide (2013) on *Adansonia digitata*, Otoide (2014), Otoide (2015) on *Afzelia africana* lend credence to this claim.

According to Saeed *et al;* (2010), the physical properties of stem and root are related to their anatomy and there is no way to interpret their role without sufficient knowledge of their structure (Otoide, 2015). This assertion is in line with the earlier reports of Luiz and William (1994) that xylem function is related to anatomy, particularly vessel element characteristics.

The present examination of vessel elements and rays in the trunk of *Theobroma cacao* seeks to abreast readers of the type, shape and class of the vessels and rays in the trunk hoping that the results will be useful and dependable in serving the interest of researchers, industries and students across the globe.

MATERIALS AND METHODS

Collection of Materials

A fully grown tree of *Theobroma cacao* within the age range of 20-25 years old was felled at the diameter at chest height (1.3meters above ground level), in cocoa plantation at Iworoko, Ado-Ekiti, Ekiti State, Nigeria. The trunk was thereafter taken to the Department of Wood Technology and Utilization (WT&U) of the Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria for identification and microscopic preparations of slides for the observations of wood vessel elements and rays.

Experimental Procedures and Maceration of Wood Samples

The procedures used in this study strictly followed Otoide (2014). The bole length of the felled tree was measured with the aid of a measuring tape from the level of chest height to the crown and the value was 1.10meters. Thereafter, transverse disc of 20cm thick axially was cut from the base, middle and the top of the trunk. A total of three transverse discs was cut out of the entire trunk. Each of the discs was divided longitudinally into two semi-circular hemispheres with the line of division passing through the pith. One of the two semi-circular hemispheres was tagged as the Northern hemisphere and the other one, the Southern hemisphere. Only the Northern semi-circular hemispheres were used for the whole of the experiments while the Southern semi-circular hemispheres were discarded. The base, middle and the top semi-circular hemispheres were further divided into three regions, with the lines of division parallel to the equator, which passes through the centre of the pith. These three regions were labeled as:

- CORE (C),
- MIDDLE (M) and
- OUTER (O).

Five blocks of the dimension, 2cm x 2cm x 2cm and another five blocks of the dimension, 2cm x 2cm x 6cm cut out of the core, middle and outer blocks earlier extracted from the three semi-circular hemispheres, each of which was cut out from the base, middle and the top of the trunk. On the base disc, five replicate extracts, each from the core, middle and the outer regions of the semi-circular hemisphere were cut out, making a total of 15 blocks of the dimension, 2cm x 2cm x 2cm and also a total of 15 blocks of the dimension, 2cm x 2cm x 6cm. A total of 30 blocks were extracted separately from the Base, Middle and the Top of the trunk. Ground total of 90 blocks of wood pellets was extracted from the whole of the tree trunk / log. All the 90 blocks of wood pellets were used for the whole of the experiments involved in the study.

Maceration of Wood Samples

In order to determine the length and diameter of the vessel elements and rays in the trunk of this species, the method outlined by Otoide (2015) was followed.

Thin slivers of wood materials were removed from the whole of the 2cm x 2cm x 2cm blocks and placed in separate test tubes containing mixture of equal amount of hydrogen peroxide and acetic acid (i.e. in ratio 1:1) individually, such that no sliver of different blocks were placed together in a test tube. The test tubes were then placed inside an electric oven for 4 hours at 80oC. The test tubes were then removed from the oven and shaken properly so as to defibrize the slivers. The test tube samples were then dropped on clean cover slides with the aid of a pipette and the slides were viewed under a calibrated microscope. Length and diameter measurements of each of the vessels and rays were averages of 50 measurements.

Preparation of Transverse Sections

In order to reveal the orientations and distributions of the vessels and rays in the trunk of the species, transverse sections (20µm thick) were cut near the centre of the core pieces with a sliding microtome (model 860; AO Spencer, Southbridge, Mass.), stained with hematoxylin-safranin, and mounted with balsam on glass slides for observation through the microscope, data collection and photomicrograph.

Experimental Design

The Experimental Design adopted for this work is a two Factorial in a Complete Randomized Design (C.R.D) with different replications of the test Samples.

FACTOR A: The longitudinal direction (Base, Middle and Top) of the trunk.

FACTOR B: The radial directions, where the sample sticks were collected (The Core, Middle and Outer) region of the trunk.

Statistical Analysis

Analysis of Variance (ANOVA) was conducted to test the relative importance of various sources of variation on the length (µm) and diameter (µm) of the vessels and rays. The main effects considered were differences along the longitudinal (i.e. Axial) and Radial Positions. The Follow up test was conducted, using Duncan Multiple Range Test (D.M.R.T). This was done to know the significant difference between the two Means at $P \leq 0.05$.

The mathematical Model for the two Factors factorial experiment is given as:
Yij = u + Ai + Bj + (AB) ij + Eij

Where:
µ = General mean of individual observation;
Ai = Effect of Factor A;
Bj = Effect of Factor B;
(AB)ij = Effect of interaction between Factor A and B;
Eij = Effect of interaction Error term.

RESULTS AND DISCUSSIONS

The results of the present evaluations are summarized in Tables 1 to 4 and illustrated in plate 1 (a-d). The total average length (µm) of the vessel elements in the trunk of *Theobroma cacao* examined was 340.69 ± 182.27. The pool means (µm) of the vessels at the base, middle and top positions of the trunk were 422.31 ± 152.20, 349.23 ± 163.23 and 250.53 ± 189. 39 respectively. Consequently, the length of vessels decreased significantly ($P \leq 0.05$) from the base to the top of the trunk (Table 1). In the same vein, examinations through the radial direction revealed 364.98 ± 139.98, 347.24 ± 182.46 and 309.86 ± 215.17 as average length (µm) of the vessels in the core, middle and outer woods of the trunk respectively. Consequently, the average length of vessels decreased insignificantly ($P \leq 0.05$) from core wood to outer wood of the trunk (Table 1).

The overall average diameter of the vessels examined in the present study was 78.62 ± 32.66. At the base, middle and top positions of the trunk, the diameter (µm) of vessels were averages of 86.34 ± 35.96, 79.24 ± 32.06 and 70.28 ± 27.96 respectively. Consequently, the diameter of the vessels decreased significantly ($P \leq 0.05$) from base to top of the trunk. In the same vein, the diameters of vessels were 82.29 ± 35.19, 75.37 ± 28.37 and 78.19 ± 34.17 for the core, middle and outer woods of the trunk. Consequently, the vessel diameters decreased insignificantly ($P \leq 0.05$) from core to outer woods of the trunk (Table2)

The rays in the trunk of the species had average height (µm) of 0.79 ± 0.47 (Table 3) and average diameter (µm) of 74.85 ± 36.68 (Table 4). Evaluation of the height of rays through the axial axes showed 1.09 ± 0.33, 0.78 ± 0.44 and 0.52 ± 0.45 as average heights of rays in the base, the middle and top of the trunk respectively. Consequently, the ray heights decreased significantly ($P \leq 0.05$) from base to top of the trunk. In the same vein, the ray heights at the radial axes were 1.03 ± 0.45, 0.66 ± 0.33 and 0.69 ± 0.51 for the core, middle and outer woods of the trunk respectively. Consequently, the heights of rays decreased significantly from core to middle of the wood and thereafter increased significantly ($P \leq 0.05$) to the outer wood of the trunk respectively (Table3). The diameter (µm) of the rays on the other hand, was 97.06 ± 32.73, 77.45 ± 34.15 and 50.05 ± 26.67 for the base, middle and top positions of the trunk respectively. Consequently, the rays decreased significantly ($P \leq 0.05$) in diameters from base to top of the trunk (Table 4). In the same vein, the average diameter of rays when examined from the radial axes were 86.09 ± 36.85, 64.78 ± 29.09 and 73.69 ± 40.54 for the core, middle and outer woods of the trunk respectively. Consequently, the diameters of rays decreased significantly ($P \leq 0.05$) from core to middle woods and thereafter increased significantly from middle to outer woods respectively (Table 4).

Microscopic observations of the vessels in transverse and tangential sections revealed both solitary and multiple vessels with lignified walls (Plate 1 a&b). The rays, on the outer hands were observed to be homogeneous and heterocellular in longitudinal and radial sections respectively (Plate 1 c & d).

Table 1: Average length (µm) of vessels in the trunk of *Theobroma cacao*

		AXIAL AXES		
RADIAL AXES	**BASE**	**MIDDLE**	**TOP**	**POOL MEAN**
CORE	345.24 ± 110.56	388.08 ± 63.28	361.62 ± 208.89	364.98 ± 139.98[a]
MIDDLE	461.79 ± 98.13	384.93 ± 172.93	194.99 ± 155.34	347.24 ± 182.46[a]
OUTER	459.90 ± 201.75	274.68 ± 200.13	194.98 ± 155.34	309.86 ± 215.17[a]
POOL MEAN	422.31 ± 152.20[a]	349.23 ± 163.23[b]	250.53 ± 189.39[c]	340.69 ± 182.27

Means with same letters in the same column are not significantly different from one another at $P \leq 0.05$.

Table 2: Average diameter (µm) of vessels in the trunk of *Theobroma cacao*

		AXIAL AXES		
RADIAL AXES	**BASE**	**MIDDLE**	**TOP**	**POOL MEAN**
CORE	86.31 ± 41.08	84.57 ± 27.63	76.01 ± 36.48	82.29 ± 35.19[a]
MIDDLE	87.87 ± 30.23	70.82 ± 28.46	67.41 ± 22.86	75.37 ± 28.37[a]
OUTER	84.84 ± 37.56	82.34 ± 38.78	67.41 ± 22.86	78.19 ± 34.17[a]
POOL MEAN	86.34 ± 35.96[a]	79.24 ± 32.06[b]	70.28 ± 27.96[c]	78.62 ± 32.66

Means with different letters in the same row are significantly different from one another at $P \leq 0.05$.

Table 3: Average height (µm) of rays in the trunk of *Theobroma cacao*

		AXIAL AXES		
RADIAL AXES	**BASE**	**MIDDLE**	**TOP**	**POOL MEAN**
CORE	1.36 ± 0.21	1.02 ± 0.30	0.72 ± 0.54	1.03 ± 0.45[a]
MIDDLE	0.80 ± 0.11	0.58 ± 0.27	0.58 ± 0.46	0.66 ± 0.33[b]
OUTER	1.09 ± 0.33	0.72 ± 0.58	0.26 ± 0.56	0.69 ± 0.51[c]
POOL MEAN	1.09 ± 0.33[a]	0.78 ± 0.44[b]	0.52 ± 0.45[c]	0.79 ± 0.47

Means with different letters in the same row are significantly different from one another at $P \leq 0.05$.

Table 4: Average diameter (µm) of rays in the trunk of *Theobroma cacao*

		AXIAL AXES		
RADIAL AXES	**BASE**	**MIDDLE**	**TOP**	**POOL MEAN**
CORE	108.36 ± 29.31	84.19 ± 26.24	65.73 ± 41.32	86.09 ± 36.85a
MIDDLE	85.85 ± 26.11	66.27 ± 29.77	42.21 ± 7.39	64.78 ± 29.09b
OUTER	96.96 ± 38.97	81.90 ± 42.97	42.21 ± 7.39	73.69 ± 40.54c
POOL MEAN	97.06 ± 32.73a	77.45 ± 34.15b	50.05 ± 26.64c	74.85 ± 36.68

Means with different letters in the same row and column are significantly different from one another at P ≤ 0.05.

Plate 1a: Multiple and solitary vessels in the trunk of *Theobroma cacao.* (Transverse view) X400.

Plate 1b: Solitary vessels in the trunk of *Theobroma cacao*. (Tangential section) X400

Plate 1c: Heterocellular ray tissues in the trunk of *Theobroma cacao* (Longitudinal section) X400.

Plate 1d: Homogeneous and heterogeneous rays in the trunk of *Theobroma cacao* (Radial section) X400

The present evaluation revealed that the vessels in the trunk are short and narrow (< 350µm). This corroborates earlier reports by Chattaway (1937) of the occurrence of short and narrow vessels in the family (Sterculiaceae) which *Theobroma cacao* belongs. This nature of vessel is a physiological advantage on this species as it will not be vulnerable to the formation of gas embolism and hence not prone to the risk of hydraulic failure in form of xylem embolism. This assertion is in line with the previous reports of Cai *et. al*. (2010), Hajek *et. al.* (2014) and Otoide (2016).

The higher length (µm) and diameter (µm) of vessels in the basal portion of this specie is a confirmation of the fact that the vessels grew and developed as the plant grows and increases in size. This pattern of vessel growth confirms the earlier reports of Baas (1982), Tyree and Zimmermann (2002) and Anfodillo *et. al.,* (2013) that mean vessel diameter in the xylem tissue generally decreases acropetally from roots to branches.

This physiological to anatomical balance might have conferred some eco-physiological advantages on this species. No wonder it thrives with low maintenance cost. The lignified wall of the vessels is a physiological advantage to the plant by providing mechanical support to it. In the same vein, the higher vessel length at the core wood region in comparison with those of the outer wood implies that the vessels grew and developed as the trunk increases in girth.

The occurrence of solitary, triple and multiple vessels as well as homogeneous and heterogeneous ray tissues in the trunk of the species confirmed the previous reports of Pandey (1978) about woody plants. The observed ray types in this specie would enable the plant to reserve much food in order to cater for the period of food recess. This assertion corroborates the claim of Metcalfe and Chalk (1989).

The nature and type of vessels and rays in this species is suspected to be contributive to the ease of penetration and diffusion of pesticides and other chemicals into the internal tissues of the species which are common day to day maintenance activities in cocoa plantations.

In view of the foregoing, it is worthwhile to suggest that the information provided in the present evaluation would be useful for anatomical, pathological and taxonomic purposes.

REFERENCES

1. Anfodillo, T; Petit, G; Crivellaro, A. (2013). Axial conduit widening in woody species; a still neglected anatomical pattern. IAWA J.34352-36410. 1163 / 22941932-00000030.
2. Baas, P. (1982). Systematic, phylogenetic and ecological wood anatomy –history and perspectives. Acta Bot. Neerl. 31501-501
3. Burkill, H. M. (2000) The usefull plants of West Tropical Africa. Vol.5, 2nd Edition; Royal Botanical Gardens, Kew, pp.364-366.
4. Cai, J.; Hacke, U; Zhang, S and Tyree, M. T. (2010). What happens when stems are embolized in a centrifuge?. Testing the cavitron theory. Physiol. Plant. 140311-32010.1111 / j.1399-3054.2010.01402
5. Chattaway, M. M. (1937). The wood anatomy of the Sterculiaceae. Philos. Trans. Bio. Soc. 228, 313-366
6. Gill, L.S, Onuja, J.E and Husaini, S.W.H. (1983) Observations on the tracheary elements of some Nigerian leguminous woods. Legume Research.6(1): 9-17
7. Gill, L. S. and C. O. Ogunlowo (1988): Histomorphology of the Tracheary Elements of some Tropical Harwoods. J. Timb. Dev. Assoc. (India), vol. XXXIV, No. 1: 42-52.
8. Hajek, P; Leuschner, C; Hertel, D; Delzon, S and Schuldt, B. (2014). Trade-offs between xylem hydraulic properties, wood anatomy and yield in populous. Tree physiol. 34744-756101093 / treephys / tpu048.
9. Irvine, F. R. (1961). Woody plants of Ghana with special reference to their uses. Oxford University Press, Amen House, London. P. 868.
10. Luiz, A.B.C.V and Williams S.C.(1994). Trunk xylem Anatomy of Mature Healthy and Blighted Grapefruit Trees on several Rootstocks. .J.Amer.Soc. Hort.Sci.119(2): 185-194
11. Metcalfe C. R. (1972). Botanical communications with special reference to plant anatomy in: G house, A.K.M and Yunus, M.(Eds.) “Research Trends in Plants Anatomy”. Tata- Mc Grew Hill, New Delhi. Pp. 7- 18.
12. Metcalfe, C. R and Chalk, L. (1989). Anatomy of the dicotyledons. Vol. 2. 2ndEdn; Wood Structure and Conclusion of the general introduction. Oxford University Press, Walton Street, Oxford OX 26DP. Pp. 297
13. Olorode, O. (2012): Taxonomy of West African Flowering Plants. CEDAR Productions, Ile-Ife, Nigeria. Pg. 45-46.

14. Otoide, J. E; Kayode, J and Afe, A. J. O. (2012). Moisture content and vessel elements in stem of Adansonia digitata Linn (Bombacaceae). Bulletin of pure and Applied Sciences. 31B(1) : 1- 9
15. Otoide, J. E. (2013). Wood density and fibre composition of the stem of *Adansonia digitata* Linn. Bulletin of Pure and Applied Sciences. Vol.32B – Botany (No. 1): P. 21-27
16. Otoide, J. E. (2014). Study of fibres in stem of Afzelia africana Sm. ex pers. Ind. J. Sci. Res. and Tech. Vol. 2 (1): 102-107
17. Otoide, J. E. (2015). Dimensions of vessels and rays in the trunk of Afzelia Africana Sm. ex. Pers. European Journal of Botany, Plant Sciences and Phytology 2 (4): 9-16.
18. Otoide, J. E. (2015).F ibre characteristics of the trunk of Citrus sinensis (L.) Osbeck. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 10 (6) Ver. III: 76-81.
19. Otoide, J. E. (2016).Axial and radial variations in wood density and moisture of the trunk of Afzelia africana Sm. ex Pers. IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) 11 (1) Ver. II: 61-65.
20. Otoide, J. E. (2016). Anatomical study of the trunk of Citrus sinensis with respect to vessels and rays. European Journal of Botany, Plant Sciences and Phytology 3 (3):1-9.
21. Pandey, B. P. (1978). Plant Anatomy. S. chard and Company Limited, Ram Nagar, New Delhi-110055. Pp. 380-381.
22. Saeed, M., P. B. Dodd and S. Lubna (2010). Anatomical studies of stems, roots and leaves of selected Citrus rootstock varieties in relation to their vigour. Journal of Horticulture and Forestry. 2(4): 87-94.
23. Tyree, M. T. and Zimmermann, M. H. (2002). Xylem Structure and The Ascent of Sap. Berlin: Springer; 10.1007 / 978-3-662-04931-0