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Full Length Research Paper

Microscopic and metrical analyses of the fibers in the woody stem of *Theobroma cacao* Linn

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Abstract

The fibers in the woody stem of *Theobroma cacao* were analyzed for length, diameter, lumen width and cell wall thickness. The analyses were along both the axial and radial axes of the stem. Measurements were in micrometers with the aid of ocular eye-piece at higher magnification. Data were collected from 50 measurements of each of the parameters and thereafter subjected to standard statistical procedures. Significant differences were determined at $P \leq 0.05$. The length and diameter of the fibers were averages of 1001.10 ± 0.25 and 19.76 ± 3.54 respectively while the lumen and cell wall thickness were averages of 11.33 ± 3.28 and 4.22 ± 1.21 respectively. In view of the results obtained in the present analyses, it was asserted that *Theobroma cacao* possessed medium sized libriform and aseptate fibers with highly thickened cell walls. It was pointed out that the mechanical strength of being able to withstand the weight, stress and strain exerted by the fruits which are borne caulifloroulsy on the stem, anthropogenic and environmental forces are not unconnected to the nature, type and sizes of fibers it possessed. The points and assertions made in the present analyses were corroborated by the views and reports of some other researchers.

Key Words: analyses, axial, diameter, fibers, length, radial, mechanical support.

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 caulifloroulsy 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, kidney and muscles and a diuretic (Ainslie). Theobromine has been proved to be poisonous to Cattle, Sheep, Pigs and Chickens (Irvine, 1961).

The woody stem of *Theobroma cacao* is one of the underutilized parts of the vegetative features. This may, to a larger extent be due to the fact that the fruits and seeds have much economic and industrial applications. This scenario has attracted much research interests on the fruits and seeds of this species over the years.

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). To this end, the present author studied the internal structure of stem of some tree plants as reported in Otoide *et al*., (2012), Otoide (2013) on *Adansonia digitata*, Otoide (2014), Otoide (2015) on *Afzelia Africana,* Otoide (2015) on *Citrus sinensis* and Otoide (2016) on *Alstonia boonei.* Additionally, the works of Gill and Okoegwale (1990) and Gill, *et. al*. (1991) are worthy of mentioning in this context.

It is interesting to note that the "highly celebrated" fruits of *Theobroma cacao* are borne caulifloroulsy on the stem. This pattern makes the stem important. Hence the fibers in the woody stem of *Theobroma cacao* are hereby analyzed.

Materials and Methods

Collection of Materials

A fully grown tree of *Theobroma cacao* which could be of about 25 years old was felled at the diameter at chest height (1.3meters above ground level), from Igbo oluwa quarters in Iworoko village, Ekiti State, Nigeria. The geographical coordinates of Iworoko village is 7^{0} 44^{1} 0^{11} North, 5^{o} 16^{1} 0^{11} East.

The log 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 for anatomical study.

The period of this research work is between the months of November, 2015 to May, 2016.

Experimental Procedures and Maceration of Wood Samples

The procedures used in this assessment strictly followed Otoide *et. al.*, (2012). 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 log. A total of three transverse discs was cut out of the entire log. 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 labelled 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 log. 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 log. 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 fibers in the Stem of this species, the method of Otoide (2014) was followed. The descriptive terms and classifications followed Metcalfe and Chalk (1989).

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 slivers of different blocks were placed together in a test tube. The test tubes were then placed inside an electric oven for 4 hours at $80^{o}C$. 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 fibers were averages of 50 measurements.

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 width (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 Discussion

Results of the present analyses were summarized in Tables 1 - 4. The overall pool means of the length (µm) and diameter (µm) of the fibers in the woody stem were 1001.10 ± 0.25 and 19.76 ± 3.54 respectively. Similarly, the overall pool means of the lumen and thickness of the cell wall (µm) were 11.33 ± 3.28 and 4.22 ± 1.21 respectively.

Table 1: Average length (µm) of fibers in the woody stem of *Theobroma cacao*

	AXIAL AXES			
RADIAL AXES	BASE	MIDDLE	TOP	POOL MEAN
CORE	660.00±0.11	1030.70±0.29	970.10±0.29	886.90±0.29c
MIDDLE	1207.50±0.19	1118.10±0.18	10005.00±0.21	1110.20±0.21a
OUTER	1000.90±0.21	1003.90±0.91	1013.50±0.20	1006.10±0.20b
POOL MEAN	956.10±0.29c	1050.90±0.23a	996.20±0.23b	1001.10±0.25

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

Analyses of lengths of fibers through the axial axes revealed pool means of 956.10 ± 0.29, 1050.90 ± 0.23 and 996.20 ± 0.23 for the base, middle and top regions of the stem respectively while through the radial axes they were 886.90 ± 0.29, 1110.20 ± 0.21 and 1006.10 ± 0.20 for the core, middle and outer regions of the stem respectively (Table 1). Similarly, the pool means of the diameter of fibers for the base, middle and top regions of the stem were 19.86 ± 3.62, 20.45 ± 3.73 and 18.97 ± 3.14 respectively while 18.87 ± 4.40, 21.13 ± 2.93 and 19.29 ± 2.70 were the fiber diameters (µm) for the core, middle and outer regions of the stem respectively (Table 2).

Table 2: Average diameter (µm) of fibers in the woody stem of *Theobroma cacao*

	AXIAL AXES			
RADIAL AXES	BASE	MIDDLE	TOP	POOL MEAN
CORE	18.48±5.27	20.55±3.94	17.57±3.46	18.87±4.40c
MIDDLE	21.21±2.76	22.27±3.28	19.90±2.30	21.13±2.93a
OUTER	19.90±1.23	18.53±3.10	19.44±3.19	19.29±2.70b
POOL MEAN	19.86±3.62b	20.45±3.73a	18.97±3.14c	19.76±3.54

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

Analyses of the width (µm) of the lumen of fibers through the axial axes resulted in pool means of 11.06 ± 3.53, 11.63 ± 2.69 and 11.63 ± 2.69 for the base, middle and top regions respectively while they were 10.71 ± 3.76, 12.67 ± 3.01 and 10.61 ± 2.57 for the core, middle and outer woods of the stem respectively (Table 3). Similarly, pool means of 4.40 ± 1.08, 4.58 ± 1.13 and 3.67 ± 1.23 were the cell wall thicknesses of the fibers in the base, middle and top regions respectively whereas, they were 4.08 ± 1.46, 4.23 ± 1.06 and 4.34 ± 1.07 for the core, middle and outer woods of the stem respectively (Table 4). Furthermore, the mean fiber diameter of 19.76 ± 3.54 in the present analyses falls within the range of 10-50µm as specified in the reports of Metcalfe and Chalk (1989) that fiber diameter varies from 10-50µm and usually about 20µm. Similarly, the cell wall thickness of 4.22 ± 1.21 as reported in the present analyses (Table 4) supported the previous reports of Metcalfe and Chalk (1989) that cell wall thickness of fibers varies considerably in different species and that it commonly ranges from 2 to 6µm and may sometimes exceed these limits.

Table 3: Average width (µm) of lumen of fibers in the woody stem of *Theobroma cacao*

	AXIAL AXES			
RADIAL AXES	BASE	MIDDLE	TOP	POOL MEAN
CORE	9.85±4.80	11.67±3.46	10.61±3.76	10.71±3.76a
MIDDLE	12.52±3.15	13.23±3.48	12.27±2.37	12.67±3.01a
OUTER	10.81±1.31	8.98±2.38	12.02±2.85	10.61±2.57a
POOL MEAN	11.06±3.53c	11.63±2.69b	11.63±2.69a	11.33±3.28

Means with the same letters in the same column are not significantly different from one another at P ≤ 0.05 while those with different letters in the same row are significantly different from one another at P ≤ 0.05.

Table 4: Average thickness (µm) of the cell wall of the fibers in the woody stem of *Theobroma cacao*

	AXIAL AXES			
RADIAL AXES	BASE	MIDDLE	TOP	POOL MEAN
CORE	4.31±1.45	4.44±1.35	3.48±1.46	4.08±1.46c
MIDDLE	4.34±0.92	4.51±1.00	3.81±1.18	4.23±1.06b
OUTER	4.55±0.82	4.77±1.06	3.71±1.05	4.34±1.07a
POOL MEAN	4.40±1.08a	4.58±1.13a	3.67±1.23a	4.22±1.21

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

The lengths and diameters of the fibers varied significantly at ($P \leq 0.05$) from base to top regions of the stem as well as from core to outer woods when analyzed from the axial and radial axes. Specifically, the fibers were more elongated and wider at the middle of the stem when analyzed through the axial and radial axes. In the same vein, the significant differences, in fiber length and diameter all through the stem depicted disproportionate growth in size both axially and radially.

Furthermore, the significant variations in the widths of the lumen depicted disproportionate growth and development of the fibers along the axial axis of the stem. However, along the radial axis they were insignificantly different ($P \leq 0.05$) from one another. This scenario depicted uniform growth and development of the fiber lumen in the core, middle and outer woods.

Furthermore, the thickness in fiber cell walls increases and varied significantly ($P \leq 0.05$) from core to outer woods. This developmental pattern is buttressed by the earlier reports of Metcalfe and Chalk (1989) that fiber cell wall thickness increases from pith outwards. There were uniform thicknesses along the axial axis from base to top of the stem as the thicknesses of the cell walls were insignificantly different ($P \leq 0.05$) from one another.

The overall mean length of 1001.10 ± 0.25 as reported in the present analyses placed the fibers in the class of medium-sized fibers according to the classification of Metcalfe and Chalk (1989). The libriform fibers were thick-walled, aseptate and with narrow lumen. It suffices to point out that the nature, sizes and type of fibers analyzed in the present work are sources of mechanical strength to *Theobroma cacao* in supporting the weight, stress and strain exerted by the fruits and leaves borne cauliflorously on the stem as well as anthropogenic and environmental forces. These points are in-line with the previous reports of Otoide (2016) about the fibers in the trunk of *Alstonia boonei* when corroborating the assertions of Metcalfe and Chalk (1989) that the mechanical strength of most dicotyledonous woody stems largely depends on the morphology of the wood fibers and their capacity to elongate by intrusive growth.

Conclusion and Recommendation

In conclusion, the present work has attempted to contribute to the scanty and obscured information on the anatomy of the woody stem of most economic tree crops in the tropics by analyzing the fibers in the stem of *Theobroma cacao* which is the main source of mechanical support to the plant.

Recommendation of rapid research into the internal structure of both economic and ordinary woods in the tropics is urgently needed to update the existing but scanty ones. This done will assist in teaching and research activities about them.

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