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ANATOMICAL STUDY OF THE TRUNK OF *CITRUS SINENSIS* WITH RESPECT TO VESSELS AND RAYS

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ABSTRACT: *Vessels and rays in the trunks of a fully grown and fruiting tree of Citrus sinensis were studied with the aid of light microscope. 50 measurements of each of the vessels and rays were carried out by using ocular micrometer to determine their lengths and diameters in micrometer (µm). The data obtained were subjected to ANOVA and Duncan multiple Range Test (D.M.R.T)to determine their average means. The average length and diameter (µm) of the vessels were 213.07±35.64 and 69.35±13. 87 respectively. On the other hand , the average height(µm) of rays was 313.65±62.02 while the diameter (µm) was averagely 44.87±23.30. From the axial direction of the trunk, the average lengths (µm) of the vessels at the base, middle and top regions were 213.38±37.72, 208.32±34.31 and 217.62±34.58 respectively, whereas, their average diameters (µm) were 76.34±10.90, 68.40±16.24 and 63.33±10.68 for the same directions and regions respectively. Similarly, the average lengths of vessels along the radial direction of the trunk were 209.97±32.03, 210.80±41.28 and 218.45±32.85 for the core, middle and outer-wood respectively whereas their average diameters were 65.55±13.42, 71.39±15.45 and 71.12±11.96 for the same direction and regions respectively. There were no significant differences (P≤ 0.05) between the average length of vessels in the base, middle and top regions as well as the vessels in the core, middle and outer-wood of the trunk respectively. However, significant differences (P≤0.05) existed between the average diameters of the vessels at the base, middle and top regions of the trunk as well as between the core, middle and outer-woods respectively. The average heights of the rays along the axial directions of the trunk did not differ significantly (P≤0.05) at the base, middle and throughout the top regions. Similarly, insignificant differences existed in the average length of rays in the core, middle and outer-wood of the trunk through the radial directions. Conversely, the diameter of the rays showed significant differences (P≤0.05) between the base, middle and top regions as well as between the core, middle and outer-wood of the trunk respectively. Structurally, transections through the trunk of this species revealed banded-multiseriate apotracheal parenchyma tissues, solitary vessels, doublet vessels elements, short and opened ended vessel elements as well as triplet vessels. The rays, on the other hand were heterogenous and properly distributed. Photomicrograghs of the anatomical features were prepared to further illustrate the type and nature of the vessels and rays observed in the trunk. It was proposed that the results obtained in the present study will be useful for researchers in the fields of Plant anatomy, Plant pathology, Plant taxonomy, Plant breeding, Plant ecology and Forestry. The results were further recommended for Pulp and Paper industries, wood construction industries and the like.*

KEYWORDS: *Citrus sinensis*, Vessels, Rays, Trunk, Axial, Radial, Anatomical, Significant Difference, Insignificant Difference.

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INTRODUCTION

Citrus sinensis, orange or sweet orange (to distinguish it from related species, such as sour orange, *C. aurantium* and mandarin orange, *C.reticulata*), is a small tree in the Rutaceae (*Citrus* family) that originated in Southern China, where it has been cultivated for millennia. Oranges, which are high in vitamins A and C and potassium, are eaten fresh or processed into juices, which can be consumed directly or further processed into concentrate, both used in numerous soda and cocktail drinks, punches, orangeades, and liqueurs (although many orange liqueurs are made from sour, rather than sweet oranges, or from a combination).

The orange tree is small, spiny tree, typically growing to 7.5m (25ft), but occasionally reaching heights up to 15m (50ft), generally with a compact crown. Leaves are leathery and evergreen, and range from elliptical to oblong to oval, 6.5-15cm and 2.5-9.5cm wide, often with narrow wings on the petioles (leaf stems). The fragrant white flowers, produced singly or in cluster of up to 6 are around 5cm wide, 5 petals and 20 to 25 yellow stamens. The fruit, which may be globose to oval, is typically 6.5 to 9.5 cm wide, and ripens to yellow to orange. The fruit skin (rind or peel) contains numerous small oil glands. The flesh or pulp of the fruits is typically juicy and sweet, divided into 10 to 14 segments (although there are seedless varieties) and ranges in color from yellow to orange to red (Vogel, 2003).

Oranges are now grown commercially worldwide in tropical, semi-tropical, and some warm temperate regions, and have become the most widely planted fruit tree in the world. In Nigeria, about 3,900,000 tonnes of *Citrus* fruits were produced from an estimated hectarage of 800,000 hectares of land in 2012 (FAO, 2012). *Citrus* is grown in the rain forest and guinea savannah. Most of these farmlands are in the remote part of the country. There are two main markets for *Citrus* fruit in Nigeria: the fresh fruit market and the processed *Citrus* fruits market (mainly orange juice) (Olife *et. al.* 2015).

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 (Beakbane and Thompson, 1939; Beakbane and Thompson, 1947; Mckenzie, 1961 and Miller *et al.*, 1961). Consequently, previous researchers such as Gill and Ogulowo (1988), Gill and Onuja (1988), Gill and Okoegwale (1990) and Gill *et. al.*(1991) reported their scientific discoveries about the fibres and other anatomical features of trunk or stem of some different woody plant species in relation to their possible end uses. More recently, Otoide (2013) reported the presence of libriform, non-septate and medium sized fibres in the stem of a fully grown species of *Adansonia digitata*. In the same vein, Sharma *et. al.* (2013) evaluated the characteristics of fibers in some weeds of Arunachal Pradesh, India for pulp and paper making. Similarly, Otoide (2014) studied the fibres in the stem of *Afzelia africana* by measuring their lengths and diameters in micrometer. He reported extremely short nature of fibres and recommends that the species be exploited for construction works and any other production in which woods with extremely short fibre length will not negatively affect the end product of productions.

According to Luiz and William (1994) xylem function is related to anatomy, particularly vessel element characteristics. General anatomical features of *Citrus* secondary xylem have been decribed by de Villiers (1939), Schneider (1968), and Webber and Fawcett (1935). In the same vein, Mendel (1945) linked xylem function and anatomy in a study of 'Shamouti'

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sweet orange transpiration. The size and number of vessel elements in the wood of trees on Palestine sweet lime (*Citrus limettioides* Tan.) were larger than in trees on sour orange (SO), a result suggesting that this difference may partly explain the greater vigor, drought tolerance, and yield of trees on sweet lime.

From the foregoing, information about the vessels and rays in the trunk of tropical woody species is very scanty and not properly documented. This present study seeks to provide additional information on the vessels and rays in the trunk of *Citrus sinensis* (sweet orange). It is envisaged that the information will be useful to researchers in the fields of Plant anatomy, Plant pathology, Plant taxonomy, Plant breeding and Forestry. Pulp and paper industries, wood industry and the like of them will as well find the results useful in handling their day to day production challenges.

MATERIALS AND METHODS

Collection of Materials

A fully grown tree of *Citrus sinensis* (Sweet Orange) within the age range of 35-40 years old was felled at the diameter at chest height (1.3meters above ground level), from an open forest in Jericho environs of Ibadan, 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 vessels 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

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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 vessels 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 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 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:

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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.

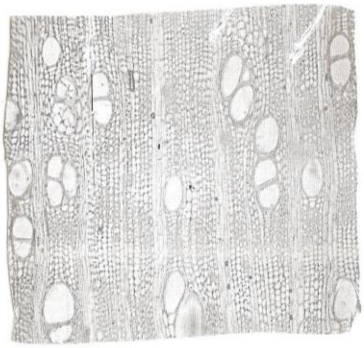

Fig. 1: Solitary, doublet, triplet vessel elements and banded-multiseriate Apotracheal parenchyma tissues in the trunk of *Citrus sinensis*. X400

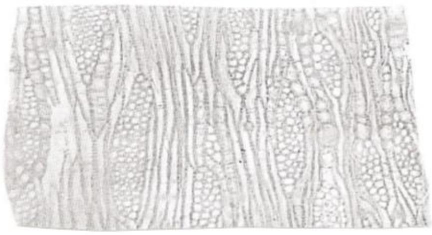

Fig. 2: Heterogeneous rays in the trunk of *Citrus sinensis*. X 400.

Fig. 3: Heterocellular rays in the trunk of *Citrus sinensis.* X 400

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TABLE 1: AVERAGE LENGTH (µm) OF VESSELS IN THE TRUNK OF *CITRUS SINENSIS*.

AXIAL AXES	RADIAL AXES			
	CORE	MIDDLE	OUTER	AXIAL MEANS
BASE	207.7 ± 30.61	213.90 ± 52.44	218.24 ± 26.81	213.28 ± 37.72[a]
MIDDLE	218.24 ± 26.81	199.64 ± 33.88	207.08 ± 40.05	208.32 ± 34.31[a]
TOP	203.98 ± 37.63	218.86 ± 34.44	230.02 ± 27.43	217.62 ± 34.58[a]
RADIAL MEANS	209.97 ± 32.03[b]	210.80 ± 41.28[b]	218.45 ± 32.85[b]	213.07 ± 35.64

Means with same letters in the column and row are not significantly different at P≤0.05

TABLE 2: AVERAGE DIAMETER (µm) OF VESSELS IN THE TRUNK OF *CITRUS SINENSIS*

AXIAL AXES	RADIAL AXES			
	CORE	MIDDLE	OUTER	AXIAL MEANS
BASE	76.10 ± 10.45	76.76 ± 13.27	76.15 ± 9.11	76.34 ± 10.90[a]
MIDDLE	55.35 ± 55.53	76.26 ± 17.18	73.58 ± 11.87	68.40 ± 16.24[c]
TOP	65.20 ± 10.59	61.16 ± 10.23	63.63 ± 11.33	63.33 ± 10.68[b]
RADIAL MEANS	65.55 ± 13.42[a]	71.39 ± 15.45[b]	71.12 ± 11.96[c]	69.35 ± 13.87

Means with different letters in the column and row are significantly different at P≤0.05

TABLE 3: AVERAGE HEIGHT (µm) OF RAY IN THE TRUNK OF *CITRUS SINENSIS*

AXIAL AXES	RADIAL AXES			
	CORE	MIDDLE	OUTER	AXIAL MEANS
BASE	318.68 ± 52.62	322.40 ± 51.21	319.30 ± 45.32	320.13 ± 48.99[b]
MIDDLE	319.44 ± 45.32	313.72 ± 69.81	302.56 ± 63.15	311.86 ± 59.70[b]
TOP	317.44 ± 89.73	293.26 ± 66.87	316.20 ± 67.74	308.97 ± 75.07[b]
RADIAL MEANS	318.47 ± 64.40[a]	309.79 ± 63.29[a]	312.69 ± 58.97[a]	313.65 ± 62.02

Means with the same letters in the column and row are not significantly different at P ≤ 0.05

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TABLE 4: AVERAGE DIAMETER (µm) OF RAY IN THE TRUNK OF *CITRUS SINENSIS*

AXIAL AXES	RADIAL AXES			
	CORE	MIDDLE	OUTER	AXIAL MEANS
BASE	52.89 ± 11.87	30.13 ± 5.14	71.92 ± 29.73	51.65± 25.20[a]
MIDDLE	71.92 ± 29.73	37.76 ± 16.47	32.98 ± 9.30	47.55 ± 26.55[b]
TOP	23.93 ± 4.20	36.27 ± 10.88	46.00 ± 10.34	35.40 ± 12.70[c]
RADIAL MEANS	49.58 ± 27.05[a]	34.72 ± 12.04[b]	50.30 ± 24.76[c]	44.87 ± 23.30

Means with different letters in the column and row are significantly different at $P \leq 0.05$.

RESULTS AND DISCUSSION

The summary of the average lengths and diameters (µm) of the vessels and rays in the trunk of *Citrus sinensis* were provided in Tables 1- 4 and further illustrated in figures 1-3.

The average lengths (µm) , diameter (µm) of vessels in the trunk of *Citrus sinensis* used for this present study were 213.07± 35.64 and 69.35 ± 13.87 respectively, while the average height (µm) and diameter(µm) of the rays were 313.65 ± 62.02 and 44.87 ± 23.30 respectively (Tables 1-4). From the axial axes, the average lengths (µm) of vessels were 213.28 ± 37.72, 208.32 ± 34.31 and 217.62± 34.58 for the base, middle and top regions of the trunk respectively whereas, they were 209.97± 32.03, 210.80±41.28 and 218.45±32.85 for the core, middle and outer woods respectively. In the same vein, 76.34±10.90, 68.40±16.24 and 63.33±10.68 were the average diameters (µm) of vessels at the base, middle and top regions of the trunks respectively, whereas, at the core, middle and outer woods of the trunk the diameter were 65.55± 13.42, 71.39±15.45 and 71.12±11.96 respectively (Table 2)

On the other hand, the average height of rays in the trunk of this economic tree (*Citrus sinensis*) were 320.13±48.99, 311.86±59.70 and 308.97±75.07 for the base, middle and top regions of the trunk respectively. Whereas, the average heights were 318.47±64.40, 309.79±63.29 and 312.69±58.97 for the core, middle and outer woods respectively (Table3). In the same vein, 51.65±25.20, 47.55±26.55 and 35.40±12.70 were the average diameter (µm) of rays for the base, middle and top regions of the trunk respectively. Whereas, in the core, middle and outer woods of the trunk, the average diameter of the rays were 49.58±27.05, 34.72±12.04 and 50.30±24.76 respectively (Table 4).

Results obtained from the study revealed that there were no significant differences ($P \leq 0.005$) in the lengths of the vessels along the axial and radial directions of the trunks as well as from the core woods to the outer woods. However, the average vessel lengths increased from the base of the trunk to the top as well as from the core wood to the outer wood of the trunk. Conversely, significant differences ($P \leq 0.05$) existed in the average diameters of the trunk. There was a consistent increase in the diameter of vessels along the axial direction from base to top of the trunk. This was however, not consistent along the radial direction of the trunk.

On the other hand, there was no significant difference ($P \leq 0.5$) in the heights of rays from base to the top of the trunk as well as from core to the outer woods of the trunk. However, the heights decreased from base to top of the trunk whereas inconsistent differences existed along

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the radial directions of the trunk. Similarly, the diameter of rays along the axial and radial directions of the trunk differ significantly ($P \leq 0.05$) from the base to top of the trunk as well as from the core to outer woods respectively. The diameters of the ray decreased from base to top of the trunk as the height of the tree increases. This however, was not consistent along the radial directions of the trunk.

Transection through the trunk revealed apotracheal parenchyma tissues consisting of banded multiseriate cells, solitary vessel elements intersperse with doublet vessel elements as well as triplet vessel elements are other anatomical features observed in the trunk of this species (Figure 1). This observation about the nature of vessels in the trunk of this species is not strange since Schneider (1968) reported same about *Citrus*. More recently the reports of Solitary and Clusters of vessels elements in the trunk of selected *Citrus* species by Luiz and William (1994) lend credence to the claim in the present study.

Consequently, it is worthwhile to opine at this juncture that the different morphological types of vessels discovered in the trunk of this species might confer some physiological and ecological advantages to it by enhancing its survival and competitive abilities as it grows in adverse or semi-friendly environmental conditions. In the same vein, a critical look at the average lengths and diameter of the vessels in the results obtained in this present study (Table 1 and 2), it is pertinent to say that the vessels in trunk of *Citrus sinensis* is short, narrow (<350um), and open-ended by semi-circular openings. This nature of vessels could be a physiological 'asset' that can enable the plant to resist negative pressure as a result of accumulation of air bubbles (embolism). The earlier reports of Okoegwale and Gill (1995) about the vessels in the woody stems of some species in the meliaceae family and the most recent reports about vessels in *Afzelia africana* by Otoide (2015) lend credence to the present views in this study. In addition to these, the openings that naturally characterized the vessels in this species are possible entry points to wood damaging insects as well as avenues for impregnation of wood with preservatives and fire retardants. Tangential and radial sections of the trunk revealed that the rays in this species were heterogeneous and heterocellular respectively (Figures 2 and 3). This nature of rays might be physiologically advantageous to the plant in boosting the food storage capacity so that it can withstand long period of food recess. Consequently, the conduction of food and water from core wood to the outer wood of this species will undoubtedly be enhanced by the type of rays revealed in the present study.

In view of the foregoing, it is pertinent to propose that the nature of the vessels and rays and their concomitant sizes and distribution patterns throughout the trunk of this species as hitherto reported will be useful information for the Plant anatomists, Plant pathologists, Plant taxonomists, Plant breeders and Foresters. In addition to this, pulp and paper industries, wood construction industries and the like will as well find the present results useful in their day to day activities.

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