COMPARATIVE MICRO-ANATOMICAL STUDIES OF THE WOOD OF TWO SPECIES OF OKRA [ABELMOSCHUS SPECIES]

[1]Osawaru, M. E., [1]Aiwansoba, R. O. and [1]*Ogwu, M. C.
[1]Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria
*Corresponding author: matthew.ogwu@uniben.edu

ABSTRACT

Okra belongs to the family Malvaceae. Common edible species are either Abelmoschus caillei [A. Chev.] Stevels or A. esculentus Moench. Seeds of the two species were obtained from the Gene bank of National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria. This study anatomically investigated the accessions to determine their distinctiveness and assess their level of diversity. Field trials were conducted at the University of Benin, Nigeria. The main stem from tagged point at seven weeks interval at three points were investigated from three dimensional views (transverse, radial and tangential section). Using light microscopy, the nature and composition of the wood were determined from the macerated part. Twenty random fibers were measured from each representative sample slide. The occurrence of the growth rings were consistent in both species showing ring porous arrangements. The vessels in A. esculentus were solitary and short radial multiples in arrangement and A. caillei were short radial multiples and irregular clusters in arrangement but both species had mainly simple perforation vessels. More so, the distribution of axial parenchyma was of paratracheal orientation. A. caillei had wide and high multiseriate rays while in A. esculentus only high multiseriate rays were observed. There was a reduction in vessel diameter and fiber length across the age in both species. Fiber diameter, fiber lumen and fiber cell wall showed different degree of fluctuations with age in both species. The study suggests that age may significantly influence anatomical characters of both species.

Keywords: Okra, Anatomy, Anatomical characterization, Light microscopy, Wood

INTRODUCTION

Okra (*Abelmoschus* species) belong to the family Malvaceae and order Malvales. *Abelmoschus* spp have diverse life forms, for instance, some may be annual herbs, perennial shrubs or trees and produce characteristic mucilaginous substance (Edwin *et al*., 2006). There are various species of the genus *Abelmoschus.* A. *caillei* [A. Chev.] Stevels and A. *esculentus* Moench are the two major cultivated species. *A. manihot* and *A. moschatus* comprise both cultivated and wild forms. The focus of this study is on two species; *A. esculentus* (common Okra) and A. *caillei* (West African Okra). The genus *Abelmoschus* is said to have originated from south and south East Asia (Hamon and Hamon, 1991; Siemonsma and Hamon, 2002) as well as somewhere around Ethiopia, with records of cultivation by ancient Egyptians in the 12th century BC from where it spread to Middle East and North Africa (Lamont, 1999). Cultivated Okra is mainly A. *caillei* or A. *esculentus*. Both are cultivated in almost same ecological zones and their uses are more or less similar. However, there are broad variation in the morphology of the genus.

Comparative anatomy assesses the similarities and differences in the histology of different species by sectioning, staining and examination under a microscope (Carlquist, 2001). When plants are to be comparatively examined for anatomical studies, it is important that similar parts of the organs are examined (Osawaru *et al.,* 2012). Stems are transversely sectioned in the middle of the internode or in addition to the node; tangentially sectioned are also examined (Carlquist, 2001; Osawaru and Abioye, 2012). Comparative wood anatomy consists of two main aspects: wood identification and evolutionary studies. Evolutionary studies can be divided into two main areas: systematics wood anatomy and ecological wood anatomy (Güvenç and Kendir, 2012; Eo and Hyun, 2013; Tiwari *et al*., 2013). An understanding of the wood properties and their variation with age provides a basis for assessing opportunities for the value-added uses. The sequence of features used by wood anatomists vary somewhat, but on the whole, the similarity of usage are more profound than the differences (Carlquist, 2001). The major sequence of features is represented as: Growth rings, vessel elements, imperforate tracheary elements (ITEs), axial parenchyma, rays and piths. Others may include idioblasts, secretory tissues, cambial variants (anomalous secondary growth), and cambial ontogeny products (Carlquist, 2001; Schweingruber *et al*., 2012).

This study aims to investigate the wood anatomical characters at different age gradation and to define the wood anatomical traits in order to contribute to their identification. In addition the study aims to determine if fibers from the wood of both species of Okra can serve as substitute raw material for pulp and paper production.

EXPERIMENTAL PROCEDURE

Seeds of both Okra species were obtained from Gene bank of National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria [Latitude 7.4^0N and Longitude 3.84^0E].

The seeds were grown on an experimental plot beside the Botanical Garden, Department of Plant Biology and Biotechnology, University of Benin, Benin City. Randomized complete block design was used with three replicates per accession. Seeds were sown directly in the soil; using two seeds per hill. Seedlings were reduced to one plant per stand two weeks after germination. Standard agronomic practices such as weeding and watering were carried out as described by Remison (2005).

The stems of the grown plants were tagged at seven weeks interval using permanent marker and seal. This process was repeated three times, that is at seven weeks (base), fourteen weeks (middle) and twenty one weeks (top). At seven weeks the stem is older and at twenty-one weeks the stem younger.

EVALUATION OF WOOD ANATOMY

The stems were harvested after twenty one weeks and sun dried. Sections within the tag region were obtained using hand saw. Samples were then stored in a polythene sac, labelled and taken to Plant Anatomy Department, Forest Research Institute of Nigeria, Ibadan for anatomical evaluation based on characters in Table 1.

Table 1: Anatomical features of A. *caillei* and A. *escuentus* examined in the study.

CODE	WOOD CHARACTER	SECTION
	Quantitative	
VTD	Vessel Tangential Diameter	Transverse
FLN	Fiber Length	Radial and Tangential
FDA	Fiber Diameter	Transverse
FCW	Fiber Cellwall	Transverse
FLM	Fiber Lumen	Transverse
	Qualitative	
VDI	Vessel Distribution	Transverse
PVE	Perforation of Vessel Elements	Radial and Tangential
FWA	Fiber Wall (thick or thin)	Transverse
GRI	Growth Rings	Transverse
RMY	Ray Morphology	Radial and Tangential
APD	Axial Parenchyma Distribution	Radial, Transverse and Tangential

SCHEDULE FOR FIBER FEATURES AND VESSEL COMPONENTS

Stem samples were boiled in water for about 2 hours to soften them and eliminate air. Each sample was then sectioned using a sliding microtome; each section was about 20 µm thick. Sections were washed with distilled water and covered with safranin stain for two minutes after which the sections were later washed with distilled water until the water became colourless. Dehydration was done by passing the wood sections through a series of bath of increasing concentrations of ethanol. Specimens were later covered with clove oil for 1 hour in order to eliminate alcohol. The sections were placed on a clean slide, excess clove oil was drained off using filter paper; a few drops of Canada balsam was added while the slide was covered with a cover slip and air bubbles were removed by applying heat gently.

For fiber features, samples were prepared into slivers of 40 mm x 3 mm x 3 mm and put in test tubes for maceration in equal volume of glacial acetic acid and hydrogen peroxide (1:1). The solution was put in the oven for 4 hours at temperature of about 100 ^{0}C for maceration. Random samples of macerated fibers were mounted on slides and examined under a light microscope.

For vessel components, each sample was prepared into slivers of about 1 mm x 2mm x 2mm. The slivers were macerated in equal volume of ethanoic acid and hydrogen peroxide (1:1) inside an oven at about 100 °C for 2hours. After this the resultant solution was agitated in order to separate it into individual fibers. Random samples of macerated fibers were mounted on slides and examined under a Zeiss light microscope.

Using a stage micrometer mounted on a Zeiss light microscope (Standard 25) under X80, random samples of macerated fibers were mounted on slides and measured. Twenty fibers were measured from each representative sample slide, following the approach employed by Jorge *et al*. (1999) when at least 20 fibers per slide were measured to keep error below 5 % for a 95 % confidence level. The microscopy was performed in accordance with the ASTM D1413-48 of 1983 and ASTM D1413-61 procedure of 2007 as well as Metcalfe and Chalk (1950; 1979)

DATA ANALYSIS

Descriptive statistical analysis (mean and standard error) was conducted on data collected using SPSS (version 20.0).

RESULTS

Anatomically, the two species of *Abelmoschus* show distinctiveness with respect to age. Results are summarized in Tables (2 - 3) and Figures (1 - 4) presented below.

Table 2: Quantitative anatomical features of the wood of *A. caillei* at three age gradation

FIBER FEATURES/MORPHOLOGY	VALUES AT DIFFERENT AGE GRADATION		
	7 WEEKS	14 WEEKS	21 WEEKS
VESSEL TANGENTIAL DIAMETER(μm) ***VTD***	90.6400 ± 5.0300	81.7600 ± 4.8400	57.0100 ± 4.7600
FIBER LENGTH (mm) ***FL***	1.1136 ± 0.0359	1.0514 ± 0.0117	1.0068 ± 0.0800
FIBER DIAMETER (mm) ***FD***	0.0231 ± 0.0016	0.0238 ± 0.0002	0.0222 ± 0.0016
FIBER CELLWALL (mm) ***CW***	0.0041 ± 0.0013	0.0052 ± 0.0019	0.0041 ± 0.0002
FIBER LUMEN (mm) ***LW***	0.0200 ± 0.0058	0.0148 ± 0.0004	0.0139 ± 0.0020

* Mean ± standard error

Table 3: Quantitative anatomical features of the wood of *A. esculentus* at three age gradation

FIBER FEATURES/MORPHOLOGY	VALUES AT DIFFERENT AGE GRADATION		
	7 WEEKS	14 WEEKS	21 WEEKS
VESSEL TANGENTIAL DIAMETER (μm) ***VTD***	88.3200 ± 7.7100	70.4000 ± 6.1000	47.99 ± 1.41
FIBER LENGTH (mm) ***FL***	0.9998 ± 0.0905	1.0379 ± 0.0237	1.0439 ± 0.0851
FIBER DIAMETER (mm) ***FD***	0.0224 ± 0.0011	0.0119 ± 0.0137	0.0223 ± 0.0028
FIBER CELLWALL (mm) ***CW***	0.0041 ± 0.0013	0.0032 ± 0.0002	0.0038 ± 0.0003
FIBER LUMEN (mm) ***LW***	0.0033 ± 0.0007	0.0154 ± 0.0002	0.0148 ± 0.0022

* Mean ± standard error

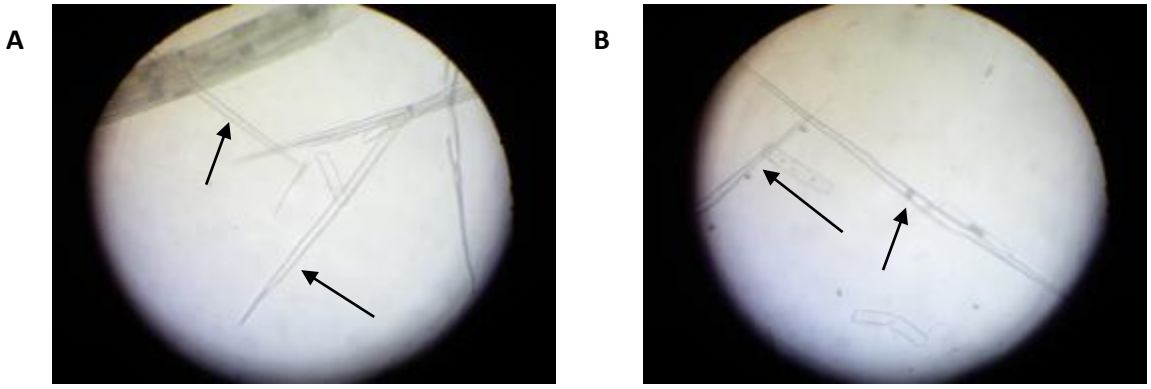
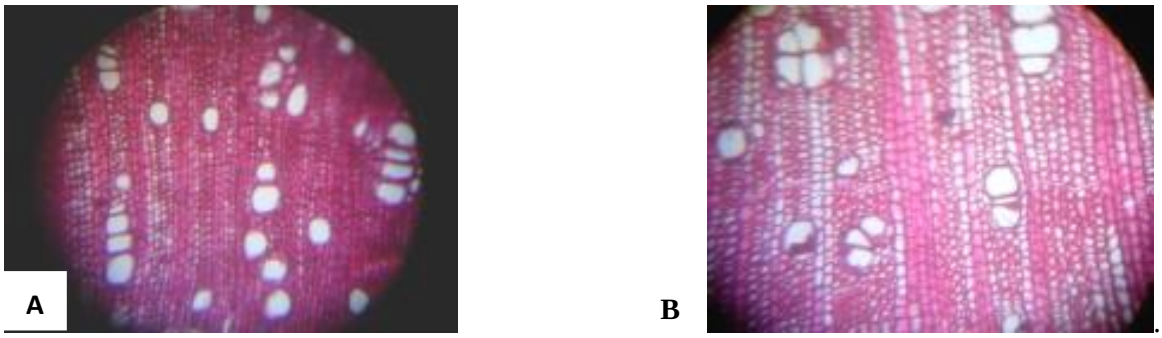
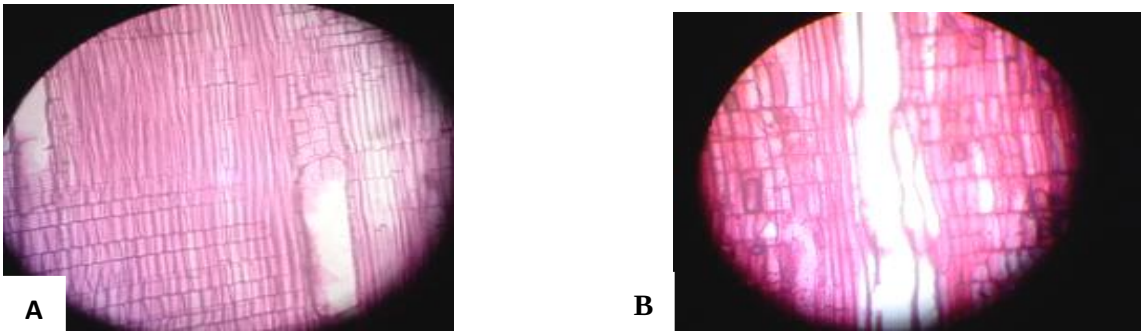


Figure 1: Fiber micrograph of (A). *A. caillei* (X80) and (B). *A. esculentus* (X80

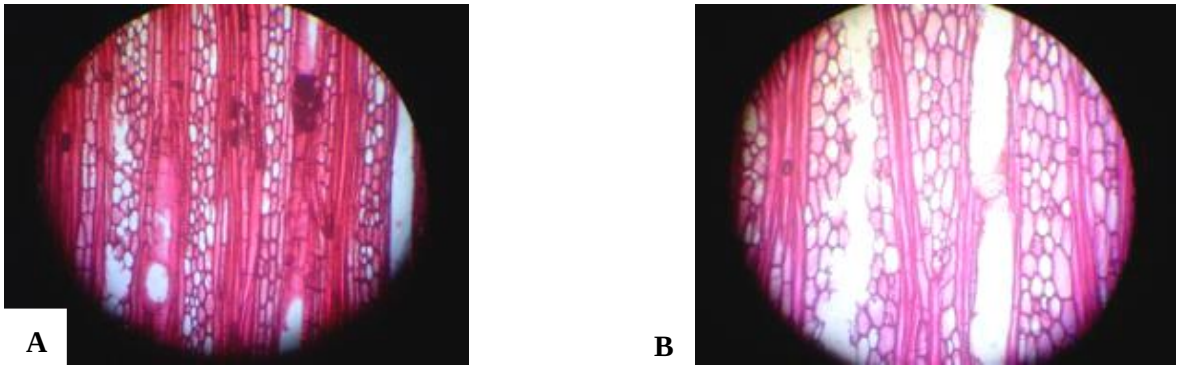
Key: **Arrow** – Fiber



Transverse view



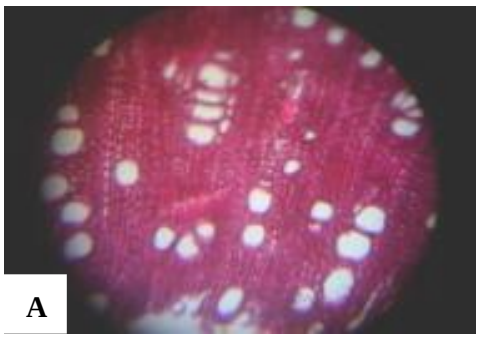
Radial view



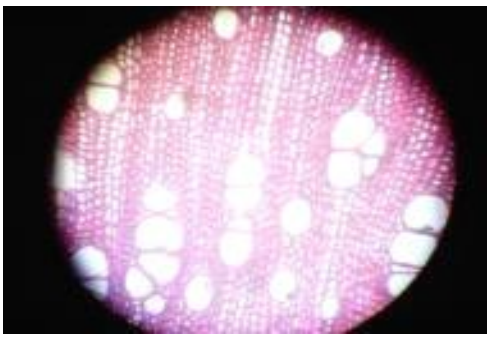
Tangential view (X80)

Figure 2: Anatomical features of both species from different view at 7-weeks gradation

Key: **A-** *A. esculentus;* **B-** *A. caillei*

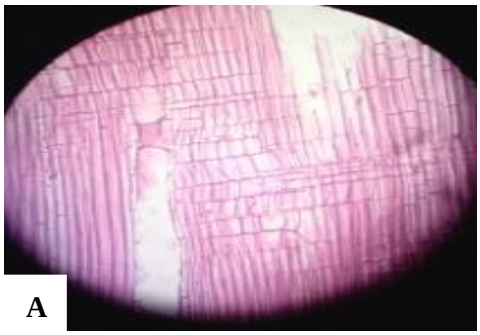


A

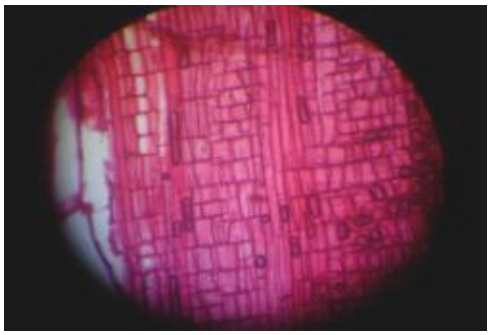


B

Transverse view (X80)

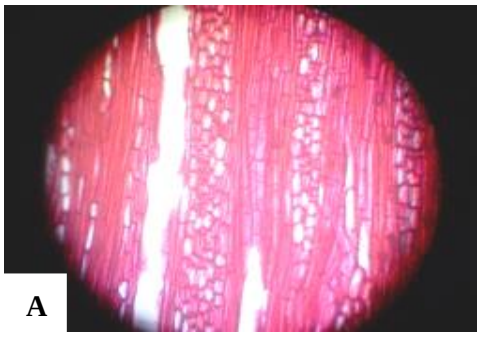


A

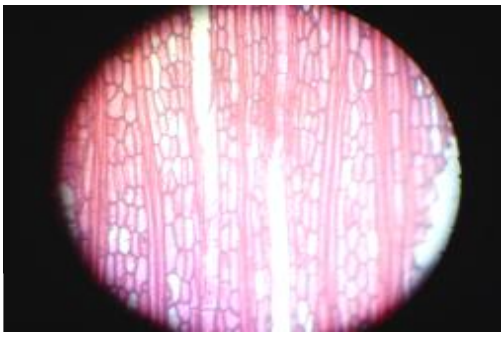


B

Radial view (X80)



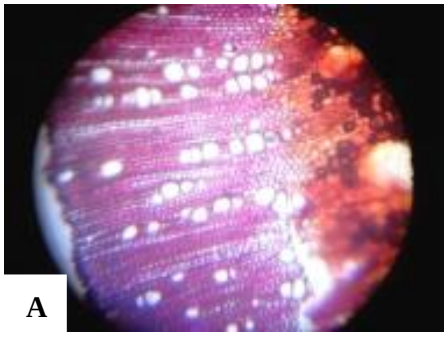
A



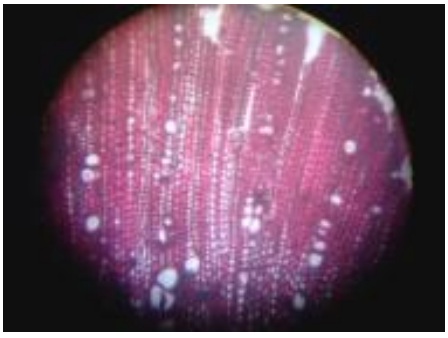
B

Tangential view (X80)

Figure 3: Anatomical features of both species from different view at 14-weeks gradation
Key: **A-** *A. esculentus;* **B-** *A. caillei*



A



B

Transverse view
Figure 4: Anatomical features of both species from transverse view at 21-weeks gradation
Key: **A-** *A. esculentus;* **B-** *A. caillei*

DISCUSSION

Anatomical structures provide evidence concerning the interrelationship of genera of uncertain taxonomic status (Metacalfe and Chalke, 1957). An understanding of wood anatomical properties and their variation with age provides a basis for assessing opportunities for value-added uses. Anatomy proves very helpful for individual identification and microscopic methods are of great value in establishing the identity of herbarium specimens which are not accompanied with flowers or fruits (Metcalfe and Chalk, 1973).

The occurrence of growth rings was consistent in both Okra species. The arrangement of vessels in both species woods shows two patterns; diffuse porous when the vessels have essentially equal diameters and are uniformly distributed and ring porous when the vessels are of unequal diameters and with largest vessels localized in the early wood (Evert, 2006). Both species shows ring porous arrangements (Figure 2 - 4). The ring-porous condition appears to be highly specialized (Evert, 2006). The vessels in *A. esculentus* are solitary and short radial multiples in arrangement while *A. caillei* are solitary, short radial multiples and irregular clusters in arrangement (Figure 2 - 4). The vessel in both species has simple perforation (Figure 2 - 4). The findings in this study coincide with the work of Metcalfe and chalk (1957) on the family Malvaceae. The patterns of distribution of axial parenchyma are of two basic types which are; apotracheal type in which the position of the parenchyma is independent of that of the vessels while in paratracheal type in which the parenchyma is associated with vessels (Evert, 2006). In this study the distribution of axial parenchyma was of paratracheal (Figure 2 - 4). Wood with paratracheal type of axial parenchyma distribution is regarded as advanced.

The rays consist of living parenchyma cells, and serve in storage and aeration. The rays may be only one cell wide (uniseriate) or several cells wide (multiseriate). The two main types of ray parenchyma cells are the procumbent (elongated in the radial plane) and upright. Base on the type of parenchyma cells present, ray can be classified as homocellular if it consists of only procumbent cells or upright and heterocellular if it consists of both cells (Evert, 2006). The entire ray system may consist of either homocellular or heterocelluar types or combinations of the two (Carlquist, 2001). The rays among five accessions are heterocellular. The variation in ray structure in different species has resulted from divergences during the evolution of xylem (Bailey, 1957; Kribs, 1935). Plants with primitive xylem have a combination of two kinds of ray, conspicuously high-celled uniseriate rays and heterocellular multiseriate rays (Evert, 2006). An advanced is characterized by possessing multiseriate, uniseriate or complete absence of rays. *A. caillei* have wide and high multseriate rays while *A. esculentus* has shown high multseriate rays though not as wide as those found in *A. caillei* (Figures 2 - 4). Radial and tangential section proves difficult at week twenty one node because it was more herbaceous than wood and the xylem has not fully differentiated. From phylogenetic consideration, both species have a common ancestor because there were no perspective differences in characters but data of this study suggests, *A. esculentus* could be more advanced than *A. caillei* regarding their wood anatomy characteristics.

The study showed that age and species could significantly influence the anatomical characters of *Abelmoschus* spp. The age variability of anatomical characters in both species is given in Table 2 and 3. The comparative analysis of the five characters (that is vessel tangential diameter, fiber length, fiber lumen, fiber diameter and fiber cell wall) shows the following result. There was variation in vessel tangential diameter in both species (Tables 2 and 3) and vessel frequency at three different age gradation in both species too (Figures 2 - 4). The vessel tangential diameter in the seven weeks node of both species was larger and vessels have thick walls and were fewer as compared to week 14 and 21 node. This variation may be due to the proportion of mature wood and juvenile wood. At the seven weeks node the wood were fully differentiated as compare with 14 and 21 weeks node. The larger the cavity, the better is the cells fitted for conduction and conversely, the thicker the walls and the smaller the cavity, the less suitable is that cell for this purpose but better is it fitted for strengthening purposes.

The vessel tangential diameter was larger in *A. caillei* than that of *A. esculentus.* These variations played significant roles in the conduction of water and nutrient for adaptive evolution of *Abelmoschus* spp. These variations in vessel size and frequency with age gradation have been reported for several plant species by numerous researchers such as Saravanan *et al.* (2013) and Carlquist (2001).

The ray width was found to show periodic changes with the three age gradation in both species but the ray width of *A. caillei* was larger as compared to that of *A. esculentus* though ray in both species was very high (Figures 2 - 4). This increase happens by anticlinal division of initial cells within rays or by merging of rays (Larson, 1994). Saravanan *et al.* (2013) has also reported similar changes in ray width with tree age in *Melia dubia* Cav. The mean value of the fiber length obtained as shown in table 2 and 3 shows variation with age and species. For *A. caillei*, the highest mean value (1.1136 mm) was obtained at seven weeks node followed by 1.0514 mm at fourteen weeks and the least 1.0068 mm at twenty-one weeks. For *A. esculentus*, the highest mean value (1.0439 mm) was obtained at twenty-one weeks followed by 1.0379 mm at fourteen weeks and the least 0.9998 mm at seven weeks. The differences in fiber length with increase in height have been reported by Emerhi (2012) to be due to the differences in the juvenile and mature as juvenile wood is expected to increase with an increase in height. Migneault *et al*. (2008) reported that fiber length and distribution play important roles in the processing and mechanical performance of fiber base products such as paper and fiber board. They also claimed that both wood density and fiber length will determine whether the quality of raw material is suitable for a specific use in paper making because of its impacts on paper characteristics. Monteoliva *et al*. (2002 and 2005) reported that fiber length greater than 1mm are preferred for pulp and paper making. The mean fiber length was greater than 1mm in *A. caillei* at the three age gradation and *A. esculentus* at all weeks 14 and 21 with these results both species may be considered as substitute raw material for pulp and paper manufacturing at different maturity phase due to variation in fiber length with age and species.

The mean value of the fiber diameter obtained as shown in Table (2 and 3) shows variation with age. For *A. caillei*, the highest mean value (0.0238 mm) was obtained at week 14 followed by 0.0231 mm at seven weeks and the least 0.0222 mm at week 21. For *A. esculentus*, the highest mean value (0.0224 mm) was obtained at week 7 followed by 0.0223 mm at week 21 and the least 0.0119 mm at week 14. The variation in fiber diameter with age may be due to uneven pattern of the plant growth. Increase in fiber diameter has been reported by Plomion *et al*. (2001) and Roger *et al*. (2007) to be due to the molecular and physiological changes that occur in the vascular cambium as well as the increase in the wood cell walls during the tree growing processes. The mean diameter both species was greater than the one reported in Jute, Hemp and Kenaf (Tahir *et al*., 2011) and in the range of Flax (Tahir *et al*., 2011) at some certain stage of maturity. Base on the results of fiber diameter, it shows that the fiber in the stem of both species may serve as a substitute for pulp and paper production within the three age gradation.

The mean value of the fiber cell wall obtained as shown in Tables 2 and 3 suggest variation with age. For *A. caillei*, the highest mean value (0.0052 mm) was obtained at fourteen weeks followed by seven and twenty-one weeks respectively. For *A. esculentus*, the highest mean value of 0.0041 mm was obtained at seven weeks followed by 0.0038 mm at twenty-one weeks and the least 0.0032 mm at week 14. The mean value which range from 0.0031 - 0.0055 mm of the fiber cell wall in both species reveal that they have thin cell wall (Figure 1) when compared with *Ficus* species (0.0019 - 0.0050 mm) reported by Ogunkunle (2010). Fiber with thin cell wall are collapsible, highly bonding, highly strength and good brightness.

The mean value of the fiber lumen obtained as shown in the Tables (2 and 3) suggests that species and age correlate with the size of fiber lumen. For *A. caillei*, the highest mean value (0.0200 mm) was obtain at seven weeks node followed by 0.0148 mm at fourteen weeks and the least 0.0139 mm at twenty-one weeks. For *A. esculentus*, the highest mean value (0.0154 mm) was obtained at fourteen weeks node followed by 0.0148 mm at twenty-one weeks and the least 0.0033 mm at seven weeks. Emerhi (2012) reported that the variation in fiber lumen with age in both species could be attributed to the increase in cell size and physiological development of the wood as the plant grow in girth. Roger

et al. (2007) reported a relationship in fiber lumen and cambium age in their study. The mean fiber lumen in this study in both species was in the range reported in *Tectonia* (Izekor and Fuwape, 2011), *Gmelina* (Ogunkunle, 2010) and greater than the one reported for indigenous hard wood species in the tropical ecosystem (Awaku, 1994). The fiber lumen in both species at different age reveal that the fiber got from their wood may compete favourably with the species prominent in pulp and paper making.

These variation in fiber properties with age played significant roles in the strength and mechanical properties of wood for different uses (Emerhi, 2012). Based on these results, the fiber of the wood of *Abelmoschus* spp may be a substitute raw material for pulp and paper manufacturing.

CONCLUSION

The anatomical studies in the three age gradation exhibited significant differences in vessel, ray and fiber features. The results from this investigation on the vessel and fiber dimension of both species stems suggest the existence of similar diversity within them. In addition to its importance on the data base of stem microanatomy, it also acts as a model study for future research on stem anatomy.

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COMPARATIVE MICRO-ANATOMICAL STUDIES OF THE WOOD OF TWO SPECIES OF OKRA [ABELMOSCHUS SPECIES]

[1]Osawaru, M. E., [1]Aiwansoba, R. O. and [1]*Ogwu, M. C.
[1]Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria
*Corresponding author: matthew.ogwu@uniben.edu

ABSTRACT

Okra belongs to the family Malvaceae. Common edible species are either Abelmoschus caillei [A. Chev.] Stevels or A. esculentus Moench. Seeds of the two species were obtained from the Gene bank of National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria. This study anatomically investigated the accessions to determine their distinctiveness and assess their level of diversity. Field trials were conducted at the University of Benin, Nigeria. The main stem from tagged point at seven weeks interval at three points were investigated from three dimensional views (transverse, radial and tangential section). Using light microscopy, the nature and composition of the wood were determined from the macerated part. Twenty random fibers were measured from each representative sample slide. The occurrence of the growth rings were consistent in both species showing ring porous arrangements. The vessels in A. esculentus were solitary and short radial multiples in arrangement and A. caillei were short radial multiples and irregular clusters in arrangement but both species had mainly simple perforation vessels. More so, the distribution of axial parenchyma was of paratracheal orientation. A. caillei had wide and high multiseriate rays while in A. esculentus only high multiseriate rays were observed. There was a reduction in vessel diameter and fiber length across the age in both species. Fiber diameter, fiber lumen and fiber cell wall showed different degree of fluctuations with age in both species. The study suggests that age may significantly influence anatomical characters of both species.

Keywords: Okra, Anatomy, Anatomical characterization, Light microscopy, Wood

INTRODUCTION

Okra (*Abelmoschus* species) belong to the family Malvaceae and order Malvales. *Abelmoschus* spp have diverse life forms, for instance, some may be annual herbs, perennial shrubs or trees and produce characteristic mucilaginous substance (Edwin *et al*., 2006). There are various species of the genus *Abelmoschus*. A. *caillei* [A. Chev.] Stevels and A. *esculentus* Moench are the two major cultivated species. *A. manihot* and *A. moschatus* comprise both cultivated and wild forms. The focus of this study is on two species; *A. esculentus* (common Okra) and A. *caillei* (West African Okra). The genus *Abelmoschus* is said to have originated from south and south East Asia (Hamon and Hamon, 1991; Siemonsma and Hamon, 2002) as well as somewhere around Ethiopia, with records of cultivation by ancient Egyptians in the 12th century BC from where it spread to Middle East and North Africa (Lamont, 1999). Cultivated Okra is mainly A. *caillei* or A. *esculentus*. Both are cultivated in almost same ecological zones and their uses are more or less similar. However, there are broad variation in the morphology of the genus.

Comparative anatomy assesses the similarities and differences in the histology of different species by sectioning, staining and examination under a microscope (Carlquist, 2001). When plants are to be comparatively examined for anatomical studies, it is important that similar parts of the organs are examined (Osawaru *et al*., 2012). Stems are transversely sectioned in the middle of the internode or in addition to the node; tangentially sectioned are also examined (Carlquist, 2001; Osawaru and Abioye, 2012). Comparative wood anatomy consists of two main aspects: wood identification and evolutionary studies. Evolutionary studies can be divided into two main areas: systematics wood anatomy and ecological wood anatomy (Güvenç and Kendir, 2012; Eo and Hyun, 2013; Tiwari *et al*., 2013). An understanding of the wood properties and their variation with age provides a basis for assessing opportunities for the value-added uses. The sequence of features used by wood anatomists vary somewhat, but on the whole, the similarity of usage are more profound than the differences (Carlquist, 2001). The major sequence of features is represented as: Growth rings, vessel elements, imperforate

tracheary elements (ITEs), axial parenchyma, rays and piths. Others may include idioblasts, secretory tissues, cambial variants (anomalous secondary growth), and cambial ontogeny products (Carlquist, 2001; Schweingruber *et al*., 2012).

This study aims to investigate the wood anatomical characters at different age gradation and to define the wood anatomical traits in order to contribute to their identification. In addition the study aims to determine if fibers from the wood of both species of Okra can serve as substitute raw material for pulp and paper production.

EXPERIMENTAL PROCEDURE

Seeds of both Okra species were obtained from Gene bank of National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria [Latitude 7.4^0N and Longitude 3.84^0E].

The seeds were grown on an experimental plot beside the Botanical Garden, Department of Plant Biology and Biotechnology, University of Benin, Benin City. Randomized complete block design was used with three replicates per accession. Seeds were sown directly in the soil; using two seeds per hill. Seedlings were reduced to one plant per stand two weeks after germination. Standard agronomic practices such as weeding and watering were carried out as described by Remison (2005).

The stems of the grown plants were tagged at seven weeks interval using permanent marker and seal. This process was repeated three times, that is at seven weeks (base), fourteen weeks (middle) and twenty one weeks (top). At seven weeks the stem is older and at twenty-one weeks the stem younger.

EVALUATION OF WOOD ANATOMY

The stems were harvested after twenty one weeks and sun dried. Sections within the tag region were obtained using hand saw. Samples were then stored in a polythene sac, labelled and taken to Plant Anatomy Department, Forest Research Institute of Nigeria, Ibadan for anatomical evaluation based on characters in Table 1.

Table 1: Anatomical features of A. *caillei* and A. *escuentus* examined in the study.

CODE	WOOD CHARACTER	SECTION
	Quantitative	
VTD	Vessel Tangential Diameter	Transverse
FLN	Fiber Length	Radial and Tangential
FDA	Fiber Diameter	Transverse
FCW	Fiber Cellwall	Transverse
FLM	Fiber Lumen	Transverse
	Qualitative	
VDI	Vessel Distribution	Transverse
PVE	Perforation of Vessel Elements	Radial and Tangential
FWA	Fiber Wall (thick or thin)	Transverse
GRI	Growth Rings	Transverse
RMY	Ray Morphology	Radial and Tangential
APD	Axial Parenchyma Distribution	Radial, Transverse and Tangential

SCHEDULE FOR FIBER FEATURES AND VESSEL COMPONENTS

Stem samples were boiled in water for about 2 hours to soften them and eliminate air. Each sample was then sectioned using a sliding microtome; each section was about 20 µm thick. Sections were washed with distilled water and covered with safranin stain for two minutes after which the sections were later washed with distilled water until the water became colourless. Dehydration was done by passing the wood sections through a series of bath of increasing concentrations of ethanol. Specimens were later covered with clove oil for 1 hour in order to eliminate alcohol. The sections were placed on a clean slide, excess clove oil was drained off using filter paper; a few drops of Canada balsam was

added while the slide was covered with a cover slip and air bubbles were removed by applying heat gently.

For fiber features, samples were prepared into slivers of 40 mm x 3 mm x 3 mm and put in test tubes for maceration in equal volume of glacial acetic acid and hydrogen peroxide (1:1). The solution was put in the oven for 4 hours at temperature of about 100 ^{0}C for maceration. Random samples of macerated fibers were mounted on slides and examined under a light microscope.

For vessel components, each sample was prepared into slivers of about 1 mm x 2mm x 2mm. The slivers were macerated in equal volume of ethanoic acid and hydrogen peroxide (1:1) inside an oven at about 100 °C for 2hours. After this the resultant solution was agitated in order to separate it into individual fibers. Random samples of macerated fibers were mounted on slides and examined under a Zeiss light microscope.

Using a stage micrometer mounted on a Zeiss light microscope (Standard 25) under X80, random samples of macerated fibers were mounted on slides and measured. Twenty fibers were measured from each representative sample slide, following the approach employed by Jorge *et al*. (1999) when at least 20 fibers per slide were measured to keep error below 5 % for a 95 % confidence level. The microscopy was performed in accordance with the ASTM D1413-48 of 1983 and ASTM D1413-61 procedure of 2007 as well as Metcalfe and Chalk (1950; 1979)

DATA ANALYSIS

Descriptive statistical analysis (mean and standard error) was conducted on data collected using SPSS (version 20.0).

RESULTS

Anatomically, the two species of *Abelmoschus* show distinctiveness with respect to age. Results are summarized in Tables (2 - 3) and Figures (1 - 4) presented below.

Table 2: Quantitative anatomical features of the wood of *A. caillei* at three age gradation

FIBER FEATURES/MORPHOLOGY	VALUES AT DIFFERENT AGE GRADATION		
	7 WEEKS	14 WEEKS	21 WEEKS
VESSEL TANGENTIAL DIAMETER(μm) ***VTD***	90.6400 ± 5.0300	81.7600 ± 4.8400	57.0100 ± 4.7600
FIBER LENGTH (mm) ***FL***	1.1136 ± 0.0359	1.0514 ± 0.0117	1.0068 ± 0.0800
FIBER DIAMETER (mm) ***FD***	0.0231 ± 0.0016	0.0238 ± 0.0002	0.0222 ± 0.0016
FIBER CELLWALL (mm) ***CW***	0.0041 ± 0.0013	0.0052 ± 0.0019	0.0041 ± 0.0002
FIBER LUMEN (mm) ***LW***	0.0200 ± 0.0058	0.0148 ± 0.0004	0.0139 ± 0.0020

* Mean ± standard error

Table 3: Quantitative anatomical features of the wood of *A. esculentus* at three age gradation

FIBER FEATURES/MORPHOLOGY	VALUES AT DIFFERENT AGE GRADATION		
	7 WEEKS	14 WEEKS	21 WEEKS
VESSEL TANGENTIAL DIAMETER (μm) ***VTD***	88.3200 ± 7.7100	70.4000 ± 6.1000	47.99 ± 1.41
FIBER LENGTH (mm) ***FL***	0.9998 ± 0.0905	1.0379 ± 0.0237	1.0439 ± 0.0851
FIBER DIAMETER (mm) ***FD***	0.0224 ± 0.0011	0.0119 ± 0.0137	0.0223 ± 0.0028
FIBER CELLWALL (mm) ***CW***	0.0041 ± 0.0013	0.0032 ± 0.0002	0.0038 ± 0.0003
FIBER LUMEN (mm) ***LW***	0.0033 ± 0.0007	0.0154 ± 0.0002	0.0148 ± 0.0022

* Mean ± standard error

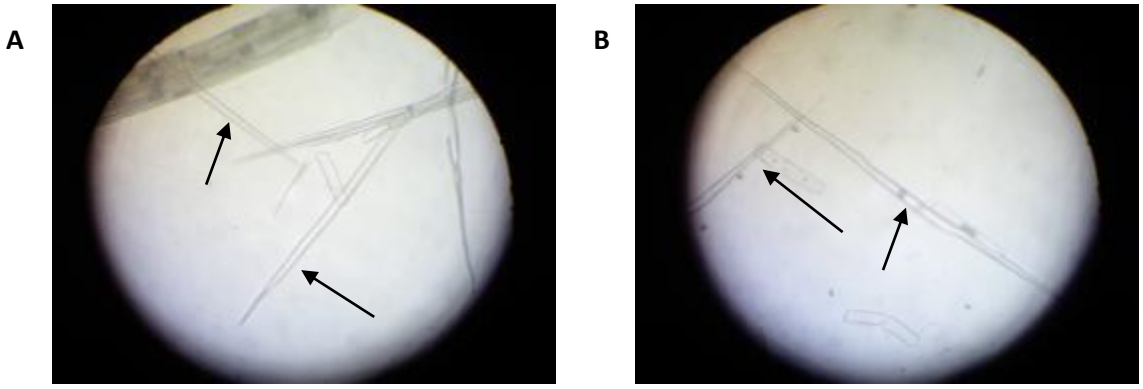
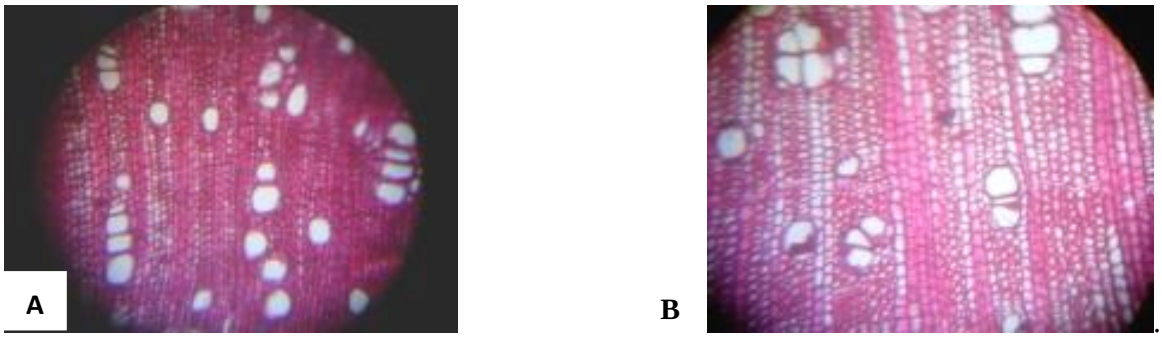
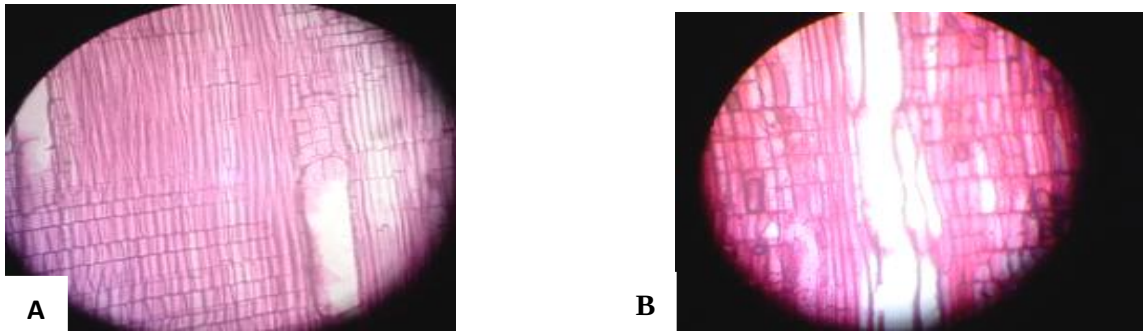


Figure 1: Fiber micrograph of (A). *A. caillei* (X80) and (B). *A. esculentus* (X80

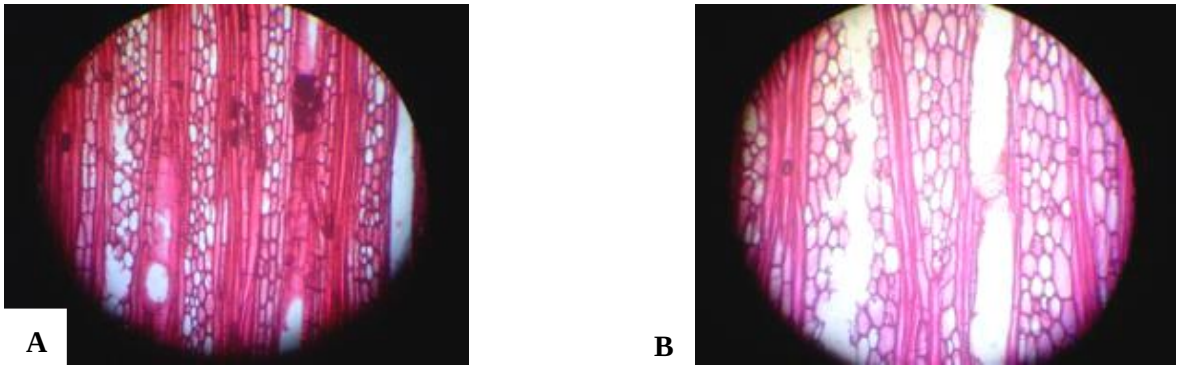
Key: **Arrow** – Fiber



Transverse view



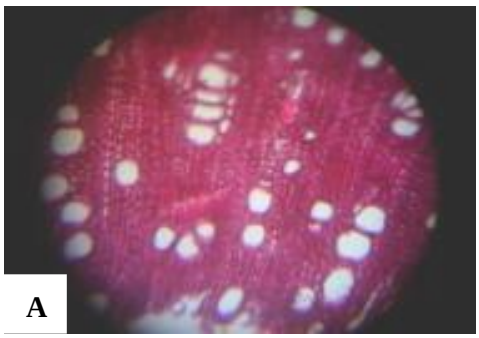
Radial view

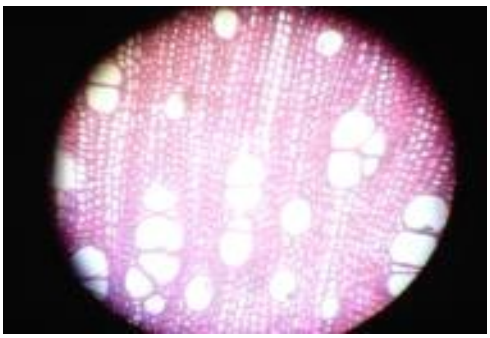


Tangential view (X80)

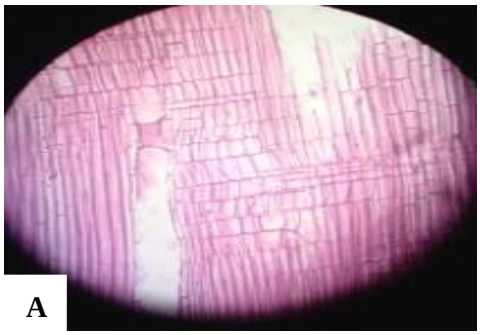
Figure 2: Anatomical features of both species from different view at 7-weeks gradation

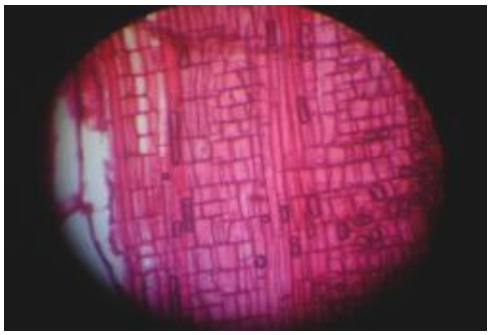
Key: **A-** *A. esculentus;* **B-** *A. caillei*



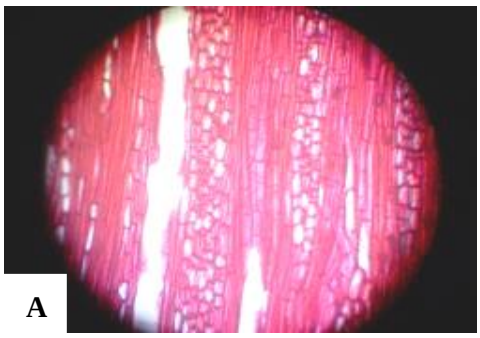


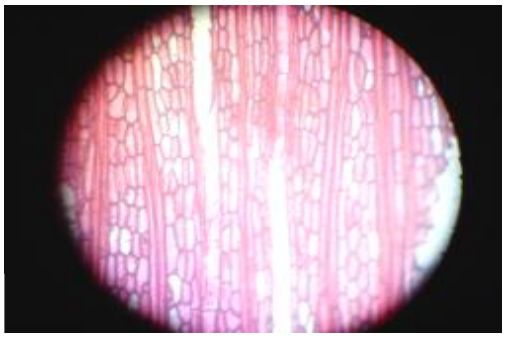
Transverse view (X80)





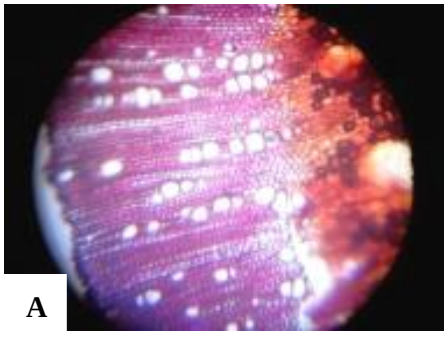
Radial view (X80)

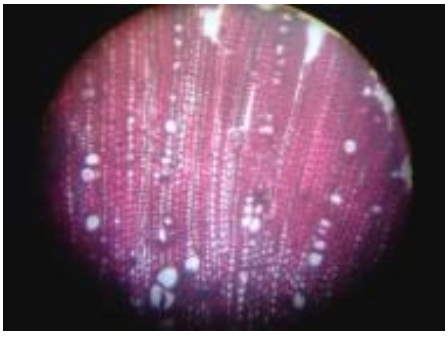




Tangential view (X80)

Figure 3: Anatomical features of both species from different view at 14-weeks gradation
Key: **A-** *A. esculentus;* **B-** *A. caillei*





Transverse view
Figure 4: Anatomical features of both species from transverse view at 21-weeks gradation

Key: **A-** *A. esculentus;* **B-** *A. caillei*

DISCUSSION

Anatomical structures provide evidence concerning the interrelationship of genera of uncertain taxonomic status (Metacalfe and Chalke, 1957). An understanding of wood anatomical properties and their variation with age provides a basis for assessing opportunities for value-added uses. Anatomy proves very helpful for individual identification and microscopic methods are of great value in establishing the identity of herbarium specimens which are not accompanied with flowers or fruits (Metcalfe and Chalk, 1973).

The occurrence of growth rings was consistent in both Okra species. The arrangement of vessels in both species woods shows two patterns; diffuse porous when the vessels have essentially equal diameters and are uniformly distributed and ring porous when the vessels are of unequal diameters and with largest vessels localized in the early wood (Evert, 2006). Both species shows ring porous arrangements (Figure 2 - 4). The ring-porous condition appears to be highly specialized (Evert, 2006). The vessels in *A. esculentus* are solitary and short radial multiples in arrangement while *A. caillei* are solitary, short radial multiples and irregular clusters in arrangement (Figure 2 - 4). The vessel in both species has simple perforation (Figure 2 - 4). The findings in this study coincide with the work of Metcalfe and chalk (1957) on the family Malvaceae. The patterns of distribution of axial parenchyma are of two basic types which are; apotracheal type in which the position of the parenchyma is independent of that of the vessels while in paratracheal type in which the parenchyma is associated with vessels (Evert, 2006). In this study the distribution of axial parenchyma was of paratracheal (Figure 2 - 4). Wood with paratracheal type of axial parenchyma distribution is regarded as advanced.

The rays consist of living parenchyma cells, and serve in storage and aeration. The rays may be only one cell wide (uniseriate) or several cells wide (multiseriate). The two main types of ray parenchyma cells are the procumbent (elongated in the radial plane) and upright. Base on the type of parenchyma cells present, ray can be classified as homocellular if it consists of only procumbent cells or upright and heterocellular if it consists of both cells (Evert, 2006). The entire ray system may consist of either homocellular or heterocelluar types or combinations of the two (Carlquist, 2001). The rays among five accessions are heterocellular. The variation in ray structure in different species has resulted from divergences during the evolution of xylem (Bailey, 1957; Kribs, 1935). Plants with primitive xylem have a combination of two kinds of ray, conspicuously high-celled uniseriate rays and heterocellular multiseriate rays (Evert, 2006). An advanced is characterized by possessing multiseriate, uniseriate or complete absence of rays. *A. caillei* have wide and high multseriate rays while *A. esculentus* has shown high multseriate rays though not as wide as those found in *A. caillei* (Figures 2 - 4). Radial and tangential section proves difficult at week twenty one node because it was more herbaceous than wood and the xylem has not fully differentiated. From phylogenetic consideration, both species have a common ancestor because there were no perspective differences in characters but data of this study suggests, *A. esculentus* could be more advanced than *A. caillei* regarding their wood anatomy characteristics.

The study showed that age and species could significantly influence the anatomical characters of *Abelmoschus* spp. The age variability of anatomical characters in both species is given in Table 2 and 3. The comparative analysis of the five characters (that is vessel tangential diameter, fiber length, fiber lumen, fiber diameter and fiber cell wall) shows the following result. There was variation in vessel tangential diameter in both species (Tables 2 and 3) and vessel frequency at three different age gradation in both species too (Figures 2 - 4). The vessel tangential diameter in the seven weeks node of both species was larger and vessels have thick walls and were fewer as compared to week 14 and 21 node. This variation may be due to the proportion of mature wood and juvenile wood. At the seven weeks node the wood were fully differentiated as compare with 14 and 21 weeks node. The larger the cavity, the better is the cells fitted for conduction and conversely, the thicker the walls and the smaller the cavity, the less suitable is that cell for this purpose but better is it fitted for strengthening purposes. The vessel tangential diameter was larger in *A. caillei* than that of *A. esculentus.* These variations

played significant roles in the conduction of water and nutrient for adaptive evolution of *Abelmoschus* spp. These variations in vessel size and frequency with age gradation have been reported for several plant species by numerous researchers such as Saravanan *et al.* (2013) and Carlquist (2001).

The ray width was found to show periodic changes with the three age gradation in both species but the ray width of *A. caillei* was larger as compared to that of *A. esculentus* though ray in both species was very high (Figures 2 - 4). This increase happens by anticlinal division of initial cells within rays or by merging of rays (Larson, 1994). Saravanan *et al.* (2013) has also reported similar changes in ray width with tree age in *Melia dubia* Cav. The mean value of the fiber length obtained as shown in table 2 and 3 shows variation with age and species. For *A. caillei*, the highest mean value (1.1136 mm) was obtained at seven weeks node followed by 1.0514 mm at fourteen weeks and the least 1.0068 mm at twenty-one weeks. For *A. esculentus*, the highest mean value (1.0439 mm) was obtained at twenty-one weeks followed by 1.0379 mm at fourteen weeks and the least 0.9998 mm at seven weeks. The differences in fiber length with increase in height have been reported by Emerhi (2012) to be due to the differences in the juvenile and mature as juvenile wood is expected to increase with an increase in height. Migneault *et al*. (2008) reported that fiber length and distribution play important roles in the processing and mechanical performance of fiber base products such as paper and fiber board. They also claimed that both wood density and fiber length will determine whether the quality of raw material is suitable for a specific use in paper making because of its impacts on paper characteristics. Monteoliva *et al*. (2002 and 2005) reported that fiber length greater than 1mm are preferred for pulp and paper making. The mean fiber length was greater than 1mm in *A. caillei* at the three age gradation and *A. esculentus* at all weeks 14 and 21 with these results both species may be considered as substitute raw material for pulp and paper manufacturing at different maturity phase due to variation in fiber length with age and species.

The mean value of the fiber diameter obtained as shown in Table (2 and 3) shows variation with age. For *A. caillei*, the highest mean value (0.0238 mm) was obtained at week 14 followed by 0.0231 mm at seven weeks and the least 0.0222 mm at week 21. For *A. esculentus*, the highest mean value (0.0224 mm) was obtained at week 7 followed by 0.0223 mm at week 21 and the least 0.0119 mm at week 14. The variation in fiber diameter with age may be due to uneven pattern of the plant growth. Increase in fiber diameter has been reported by Plomion *et al.* (2001) and Roger *et al*. (2007) to be due to the molecular and physiological changes that occur in the vascular cambium as well as the increase in the wood cell walls during the tree growing processes. The mean diameter both species was greater than the one reported in Jute, Hemp and Kenaf (Tahir *et al*., 2011) and in the range of Flax (Tahir *et al*., 2011) at some certain stage of maturity. Base on the results of fiber diameter, it shows that the fiber in the stem of both species may serve as a substitute for pulp and paper production within the three age gradation.

The mean value of the fiber cell wall obtained as shown in Tables 2 and 3 suggest variation with age. For *A. caillei*, the highest mean value (0.0052 mm) was obtained at fourteen weeks followed by seven and twenty-one weeks respectively. For *A. esculentus*, the highest mean value of 0.0041 mm was obtained at seven weeks followed by 0.0038 mm at twenty-one weeks and the least 0.0032 mm at week 14. The mean value which range from 0.0031 - 0.0055 mm of the fiber cell wall in both species reveal that they have thin cell wall (Figure 1) when compared with *Ficus* species (0.0019 - 0.0050 mm) reported by Ogunkunle (2010). Fiber with thin cell wall are collapsible, highly bonding, highly strength and good brightness.

The mean value of the fiber lumen obtained as shown in the Tables (2 and 3) suggests that species and age correlate with the size of fiber lumen. For *A. caillei*, the highest mean value (0.0200 mm) was obtain at seven weeks node followed by 0.0148 mm at fourteen weeks and the least 0.0139 mm at twenty-one weeks. For *A. esculentus*, the highest mean value (0.0154 mm) was obtained at fourteen weeks node followed by 0.0148 mm at twenty-one weeks and the least 0.0033 mm at seven weeks. Emerhi (2012) reported that the variation in fiber lumen with age in both species could be attributed to the increase in cell size and physiological development of the wood as the plant grow in girth. Roger *et al*. (2007) reported a relationship in fiber lumen and cambium age in their study. The mean fiber

lumen in this study in both species was in the range reported in *Tectonia* (Izekor and Fuwape, 2011), *Gmelina* (Ogunkunle, 2010) and greater than the one reported for indigenous hard wood species in the tropical ecosystem (Awaku, 1994). The fiber lumen in both species at different age reveal that the fiber got from their wood may compete favourably with the species prominent in pulp and paper making.

These variation in fiber properties with age played significant roles in the strength and mechanical properties of wood for different uses (Emerhi, 2012). Based on these results, the fiber of the wood of *Abelmoschus* spp may be a substitute raw material for pulp and paper manufacturing.

CONCLUSION

The anatomical studies in the three age gradation exhibited significant differences in vessel, ray and fiber features. The results from this investigation on the vessel and fiber dimension of both species stems suggest the existence of similar diversity within them. In addition to its importance on the data base of stem microanatomy, it also acts as a model study for future research on stem anatomy.

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RAPD PCR GENOTYPING OF TEN ACCESSIONS OF TOMATO (*Lycopersicon esculentum* Con. [Mill.] Syn. *Solanum lycopersicon* [Linn.])

***[1]Ogwu, M. C., [1]Chime, A. O., [1]Osawaru, M. E., [1]Aiwansoba, R. O. and [1]Gbadebo, F.**
[1]Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria
*Corresponding author: matthew.ogwu@uniben.edu

ABSTRACT

Tomato (Lycopersicon esculentum Con. [Mill.] Syn. Solanum lycopersicon [Linn.]) belongs to the family Solanaceae and order Solanales. The plant originated from South America but has been domesticated worldwide. This study investigated the variation in Tomato germplasm using RAPD PCR genotyping technique. Seven accessions; NGB01254, NGB01250, NGB01302, NG/DE/MAR/09/019, NGB01301, NGB01255 and NGB 01237 were collected from Gene Bank while three accessions were obtained from home gardens including DE/IK-L-001, DE/IK-L-002 and ED/EG-L-001. The samples were cultivated in a Screen House in the University of Benin, Benin City, Nigeria. Standard procedures were used for DNA extraction and purification while the commercial primer OPA-09 (5[1]–GGG TAA CGC C-3[1]) was employed for genotyping. Each DNA fragment generated from RAPD PCR genotyping was treated as a separate character and scored as a discrete variable, which was used to distinguish the accessions. Results suggest the primer was able to amplify less than 50 % of the samples studied but was able to amplify more than 50 % of the collections from home gardens. Three genetic types were observed, which may be related to nutrients, growth condition and species of Tomatoes studied. Of the ten samples studied, 4 (40.0 %) were amplified and typeable with the OPA 09 commercial primer while the remaining 6 (60.0 %) were not typeable with this primer. Although this study have further enumerated the advantages of molecular markers in plant characterization, it is important that a combination of molecular and morphological traits be considered for effective characterization of plant germplasm.

Keywords: Vegetable, DNA, Genotyping, Plant Characterization and Conservation

INTRODUCTION

Tomato (*Lycopersicon esculentum* Con. [Mill.] Syn. *Solanum lycopersicon* [Lin.]) most likely originated in western part of South America as the origin of domestication is still an issue of debate. Two hypotheses based on botanical evidence and molecular studies have been postulated for the origin of domestication suggesting Peru but there is no archeological proof to support the evidence (de Candolle 1882; Miller, 1940; Luckwill, 1943; McMeckin, 1992; Nesbitt and Tansley, 2002). Another hypothesis is based on the fact that there is no evidence of pre-Colombian cultivation of Tomato in Mexico (Jenkins, 1948). However, the domestication of Tomato remains unresolved since evidence given are not conclusive enough to show origin of domestication (Peralta and Spooner, 2007). The Tomato plant was introduced into various parts of Africa by early European visitors and is widely cultivated and used.

Tomato belongs to the family Solanaceae also called nightshade family. Carolus Linnaeus placed it in the genus *Solanum* as *Solanum lycopersicon*. In 1768, Miller placed it in a new genus *Lycopersicum* as *Lycopersicum esculentum*. This naming was based on morphological analysis (Luckwill, 1943; Child, 1990). Genetic analysis later confirmed that Linnaeus classification was correct and has recently been reintegrated into the genus *Lycopersicon* (Bohs and Olmstead, 1997; Peralta and Spooner, 2001).
The characterization of Tomato is important particularly in crop improvement and breeding. Characterization is the description of plant germplasm to provide information on the traits of accessions. It employs markers to facilitate easy and fast description of phenotype, grouping of accessions, understanding of species adaptation as well as retrieval of valuable germplasm for

breeding programs. More so, it promotes a better understanding about composition of the collections, their genetic diversity and aid documentation of the genetic variability in a population. Recently, several studies have been carried out with the aim of evaluating plant characteristics including Osawaru *et al.* (2012); Mezette *et al.* (2013); Osei *et al*. (2014). However, a more accurate approach using molecular marker technique is required to establish this objective (O'Donnell *et al.*, 2008). Molecular markers refer to assays that allow the detection of specific sequences between two or more individuals and are important for studying the organization of plant genome (Modini *et al*., 2009). Molecular markers are very important tools for identification of genotypes in studying the organization and evaluation of plant genome. With developments in molecular technique, large number of DNA markers such as RFLP, SSR, CAP, EST and RAPD markers have been developed and used for the identification of genetic polymorphism (Singh *et al.*, 2007).

The characterization of plant germplasm with molecular markers has many advantages; they are less affected by environment, subjected to rapid detection and are not pleiotropic. Of all genetic molecular marker developed so far, RAPD is the most widely used, for identification of relationships amongst species and between cultivars (Singh and Pal, 2013; Sharifova, 2013). Stand out advantages of using RAPD is that it requires small amount of DNA, short primers of arbitrary sequence, easy, fast and highly cost effective (Welsh and McClelland, 1990).
Hence, this study aims to investigate the variation and diversity in Tomato germplasm obtained from gene bank and locally using RAPD genotyping technique. This will aid an understanding of the relevant methods of conservation and seed storage for this essential crop and contribute to Tomato database in Nigeria.

MATERIALS AND METHODS

STUDY AREA: The samples were planted in the Screen House, Department of Plant Biology and Biotechnology, University of Benin, Benin City [6.20 ^{o}N and 5.37 ^{o}E] located within the tropical rain forest zone.
SOURCE OF SEEDS: Seven accessions; NGB01254, NGB01250, NGB01302, NG/DE/MAR/09/019, NGB01301, NGB01255 and NGB01237 were collected from the Gene Bank of the National Center for Genetic Resources and Biotechnology, Ibadan, Nigeria. Three local accessions were obtained from home gardens including DE/IK-L-001 and DE/IK-L-002 from Agbor, Delta State and ED/EG-L-001 Uselu, Edo State.
PLANTING: Prior to planting, soil samples were collected, air dried and filtered to remove soil particles and debris. Nursery pots were slightly perforated to allow drainage. 0.75 kg of soil samples were weighed into bowls. Six seeds per hole were sown at a depth of 2 cm into the soil and later thinned to three. Plants were watered every day. Fresh leaves of Tomato were harvested 16 weeks after sowing and taken to the laboratory for DNA extraction and RAPD PCR genotyping.

DNA EXTRACTION

DNA extraction and purification was done using ZR PLANT SEED DNA Miniprep^{TM}50 Preps model D6020 (Zymo Research, California, USA). 150 mg of the Tomato was transferred to ZR BashingBead^{TM} Lysis tube. 750 µL lysis solution was added to the tube. The bead containing the solution was secured in a bead beater fitted with a 2 ml tube assembly and processed at maximum for 5 minutes. The ZR Bashing Bead^{TM} Lysis tube was centrifuged in a micro centrifuge at 10,000 x g for 1 min 400 µL of the supernatant was pipetted into a Zymo-Spin^{TM} IV Spin Filter in a collection Tube and centrifuged at 7000 x g for 1 min. This was followed by the addition of 1,300 µL of seed/plant DNA Binding Buffer into filtrate in the Collection Tube. Afterwards, 800 µL of the mixture was transferred into a Zymo-Spin^{TM} IIC Column in a collection Tube and centrifuged at 10,000 x g for 1 min. The flow through was discarded from the Collection Tube and the process was repeated to obtain the remaining products. The 200 µL DNA Pre Wash Buffer was added into the Zymo-Spin^{TM} IIC Column DNA Pre-Wash Buffer was added into the Zymo-Spin^{TM} IIC Column in a new collection tube and centrifuged at 10,000 x g for 1 min. This was followed by the addition of 500 µL. Plant DNA Wash Buffer into the Zymo-Spin^{TM} IIC Column and centrifuged at 10,000 x g for 1 min. The zymo-Spin^{TM} IIC Column was transferred into a clean 1.5 ml microcentrifuge tube and 100 µL of DNA

Elution Buffer was then added directly to the Column matrix. The DNA obtained was used as a template during the assay.

DNA AMPLIFICATION USING RAPD-PCR AND ELECTROPHORESIS

Randomly amplified polymorphic DNA (RAPD PCR) was performed according to the method of (Hsueh *et al.*, 2002) DNA amplification was carried out by using 20 µL reaction mixture containing 16.5 µL sterile ultra-pure water and bead that contains 2.5 uL of IX PCR Buffer, 1.5 µL $MgCl_2$ (50 mmol/L), 1.0 µL dNTP (0.2 mmol/L), 0.5 µL OPA- 09 primer (0.4 µmol/L), 0.25 µL Platinum Taq DNA polymerase (0.5U) (Promage) and 3 ng DNA. The commercial primer OPA-09 (5^1 –GGG TAA CGC C-3^1) was used for genotyping based on the better discriminatory power observed in previous studies. Amplification was performed in a DNA thermocycler (a & e laboratory model 005 gradient 48 well PCR) programmed for initial denaturing at 94 ºC for 1 minute, annealing at 42 ºC for 2 mins, at 72 ºC for 10 mins for final extension. The number of cycles repeated was 30. A negative control without a DNA template was included in each RAPD- PCR run. Amplified products were separated using 1.5 % agarose gel electrophoresis in TAE buffer (40 Mm Tris- acetate, 2 Mm EDTA [pH 8.3] Performed at 70 V for 2.5 hrs. Gels were stained with 0.5 µg/ml of ethidium bromide and then visualized. The electrophilic profiles were observed visually. Only the major amplicons and consistent minor bands were considered in the analysis. Stained gels were examined under ultra-violet trans illuminator in a photo documentation system. A DNA ladder digest of 1 kb (Fermenters USA) was used as molecular weight marker.

DATA ANALYSIS

Each DNA fragment generated from PCR genotyping was treated as a separate character and scored as a discrete variable using 1 to indicate presence and 0 for absence of band. Similarities between similar specifics were scored based on number of bands and band sizes.

RESULTS

The results of PCR genotyping are presented in Plates 1a, 1b, 2a, 2b and Tables 1 and 2

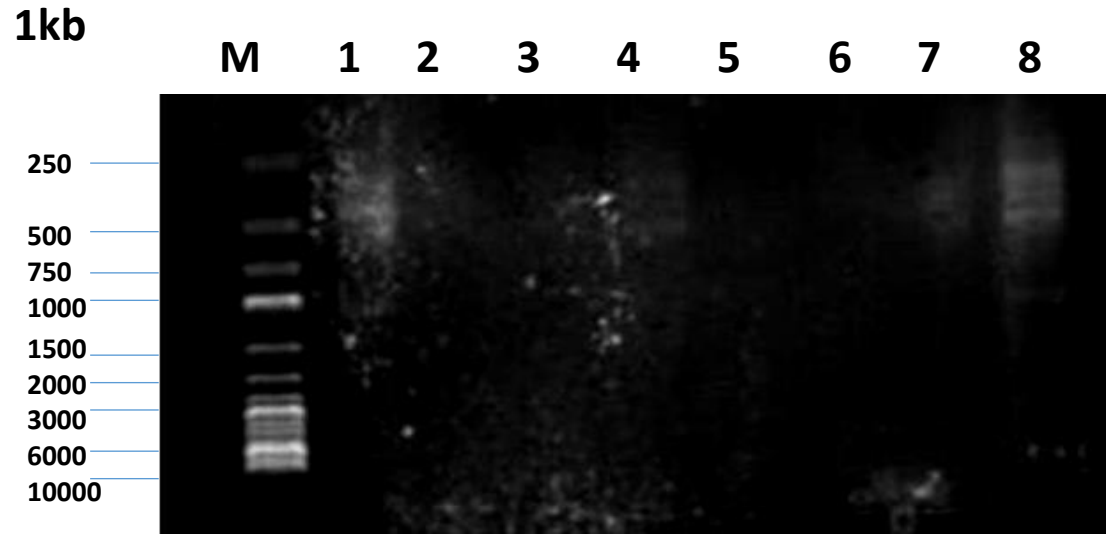

Plate 1a: Amplification pattern for the leaf samples.
M is 1kb DNA Marker.

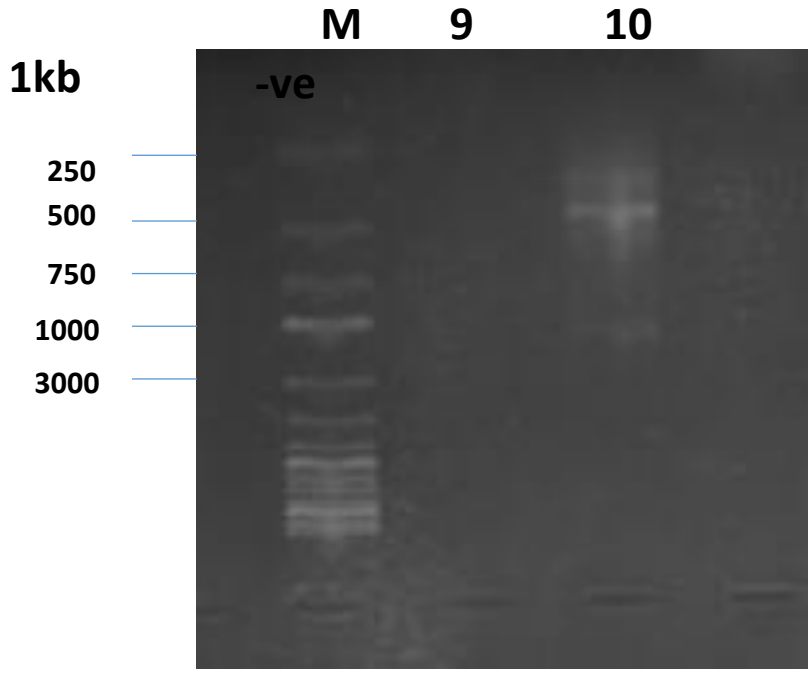

Plate 1b: Amplification pattern for the leaf samples.
M is 1kb DNA Marker.

Key:
1 = NGB01254, 2 = NGB01250, 3 = NGB01302, 4 = NG/DE/MAR/09/019, 5 = NGB01301, 6 = NGB01255, 7 = NGB01237, 8 = DE/IK-L-001, 9 = DE/IK-L-002 and 10 = ED/EG-L-001

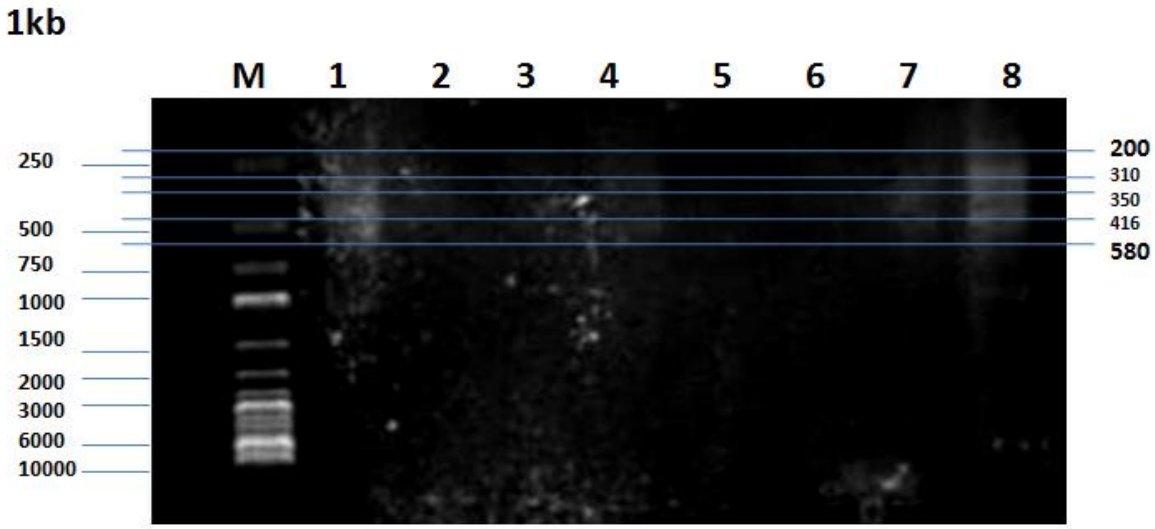

Plate 2a: Calculation of the amplified band sizes with corresponding DNA marker.

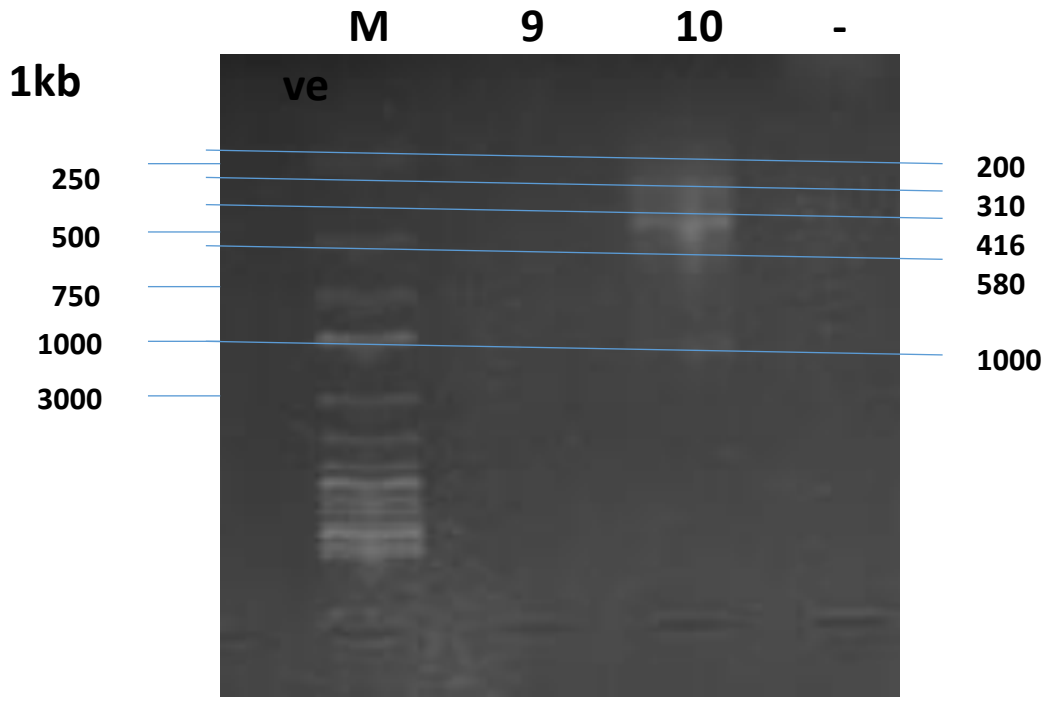

Plate 2b: Calculation of the amplified band sizes with corresponding DNA marker.

Key:
1 = NGB01254, 2 = NGB01250, 3 = NGB01302, 4 = NG/DE/MAR/09/019, 5 = NGB01301, 6 = NGB01255, 7 = NGB01237, 8 = DE/IK-L-001, 9 = DE/IK-L-002 and 10 = ED/EG-L-001

Table 1: Detection of amplification (genetic) band on leaves of Tomatoes accessions after RAPD-PCR amplification with OPA 09 Primer

		Samples			
DNA Marker (Major bands)	**Detected band levels**	**1**	**4**	**8**	**10**
10,0000	-	-	-	-	-
6,000	-	-	-	-	-
3,000	-	-	-	-	-
1000	**1000**	**0**	**0**	**0**	+
750		-	-	-	-

	580	**0**	**0**	+	+
500	-	-	-	-	-
	416	+	+	+	+
	350	+	+	+	+
	310	+	+	+	+
250					
	200	+	+	+	+

Key:

1 = NGB01254, 4 = NG/DE/MAR/09/019, 8 = DE/IK-L-001, 10 = ED/EG-L-001

+ = Presence - = Absence

Table 2: Occurrence of bands and frequency

Samples Numbers N = 4	Number of bands	Samples N = 10	Number of bands observed	**Frequency**	**Percent (%)**
1	4				
4	4		4	2	50
8	5		5	1	25
10	6		6	1	25
		Total Number of samples typeable= 4 (40.0%)	Number of genetic type =3		
		Total			**100 %**

Key:
1 = NGB01254, 4 = NG/DE/MAR/09/019, 8 = DE/IK-L-001, 10 = ED/EG-L-001

DISCUSSION

Tomato genotyping have been done in this study using RAPD technique. RAPD markers have been applied in gene mapping, population genetics, molecular evolutionary genetics and plant breeding because they are simple, cheap, fast and effective. This study was done to determine the genetic fingerprints of Tomato accessions sampled from gene bank and home gardens. Results suggest adequate protection is necessary for accessions sampled from home gardens in Edo and Delta states as well as the plant breeders' rights. Molecular fingerprinting of plant variety is importance for protecting plant breeders' rights as well as for the identification of hybrids from their respective parents (Pal and Singh, 2013).

RAPD-PCR method provides a direct analysis of the genome not possible with morphological methods and therefore, serves as a powerful tool for biosystematics studies (Taylor-Grant and Soliman, 1999). The technique does not require previous knowledge of the target genome and is relatively simple and rapid to carry out, hence, applicable in population genetics, analyses of biodiversity and studies of relationships among species (Ayten *et al*., 2009).

Commercial OPA -09 primer was used due to the high discrimination power in genotyping Tomatoes in previous studies (Pal and Singh, 2013). The OPA 09 primer showed bands which can be used to distinguish between the plant hybrids. This is similar to previous works (Schnell *et al.,* 1999; Pal and Singh, 2013). The primer was able to amplify less than 50 % of the samples studied (NGB01254, NG/DE/MAR/09/019, DE/IK-L-001 and ED/EG-L-001) suggesting that more commercial OPA primers should be tested on the leaf samples. This will highlight the most appropriate primer for genotyping tomatoes accessions. Interestingly, in this study, the primer was able to amplify more than 50 % of the collections from home gardens including DE/IK-L-001 and ED/EG-L-001. Three genetic types were observed, variation may be related to nutrients, growth condition and species of tomatoes studied. Of the nine samples studied, 4 (44.4 %) were amplified and typeable with the OPA 09 commercial primer. OPA-9 was a convenient polymorphic marker in the present study, which was discernible with the appearance and disappearance of DNA bands suggesting a high degree of potential variation.

The remaining 5 (55.5 %) were not typeable with this primer. Of the four samples that showed amplifications, two had four bands occurring between 200 - 416 bp showing that they most likely possess and/or belong to the same genotype. One had five bands ranging between 200 – 580 bp belonging to another genotype. Another sample had six bands ranging from 200 – 1000 bp showing that they two may also belong to another different genotype. In all three different genotypes were observed. In addition, the leaf samples were harvested and kept in a polythene sack for two days and then stored in a -30 ^{o}C freezer prior to DNA extraction, which may have affected the yield of DNA obtained from the various leaves. More so, it was difficult to macerate the leaves after it had absorbed liquid. In addition, DNA extraction was done by a commercial Kit. There is a need to compare yield with other extraction method like boiling or Phenol: chloroform methods.

Ayten *et al.* (2009) opined that species identification should not rely only on RAPD because it may not be a reliable method of identification alone, rather it works well in combination with other strong co-dominant methods. Furthermore, the relationships established by electrophoretic profile of RAPD do not match those established relationships based on morphological characters as RAPD seems to show more variation than morphological analysis, which can be explained by considering the advantage of representing full genome of a species unlike morphological differences which are more dependent on environmental conditions (Singh *et al*., 1994). The study of Ezekiel *et al.* (2011) suggests the use of RAPD was very effective in classifying tomato accessions genotypically and that eco-geographical differences may not necessarily determine the distribution and diversity in Tomato crop in Nigeria.

Hence, they suggested that characterization of Tomato be based on a combination of molecular and morphological traits to reliably determine the differences among the Tomato varieties and eliminate the error of assigning different names to the varieties or vice-versa.

In conclusion, advancement in the field of systematics have promulgated the use of molecular techniques to classify, identify, describe and name plant species against the use of changeable characters. This study has contributed to the knowledge base of Tomato in Nigeria and suggests the use of more samples and additional RAPD-PCR primers as well as the different types of molecular markers techniques with more reliability than RAPD-PCR technique. This is necessary because RAPD-PCR can give inconsistent results (Karp *et al.* 1997), do not detect co-dominance and therefore cannot measure allelic frequency and results are not always comparable between laboratories and sometimes even between experiments (Hodgkins, 2001).

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