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Research Article
Anatomical Characterization of Representative Members of Different Groups of Tracheophytes Found in Rivers State, Nigeria

M.G. Ajuru and F.W. Nmom

Department of Plant Science and Biotechnology, Faculty of Science, Rivers State University, Nkpolu-Oroworukwo, P.M.B. 5080, Port Harcourt, Rivers State, Nigeria

Abstract
Background and Objective: Tracheophytes are group of plants characterized by the presence of vascular bundles; xylem and phloem. There are three groups of tracheophytes; Pteridophytes, Gymnosperms and Angiosperms. The main purpose of this study was to anatomically characterize representative members of the three groups of the vascular plants to show the evolutionary relationship among them. **Materials and Methods:** Fresh samples of the species were collected from different parts of Rivers state, fixed in Formalin, Acetic Acid (FAA) and alcohol, dehydrated in alcohol series, hand sectioned, stained with alcian blue and counter stained with safranin. **Results:** Results showed increasing number of vascular bundles from the lowest to highest group of plants. Amphicribral bundles were present in the Sword fern and collateral and open bundles in Sago palm, Caribbean Pine, African Jointfir and waterleaf, while there were scattered and closed bundles in day flower. The arrangement of vascular bundles were concentric in the ferns, omega shaped in Sago palm, found in a ring in the Pine, African Jointfir and waterleaf and scattered in the day flower, with vessel elements present in only Gnetum and the Angiosperms. **Conclusion:** The similarities among these group of plants point towards the same evolutionary origin while, the differences indicated the different level of evolutionary development among the plants.

Key words: Pteridophytes, gymnosperms, angiosperms, vascular bundles, evolutionary relationship

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Corresponding Author: M.G. Ajuru, Department of Plant Science and Biotechnology, Faculty of Science, Rivers State University, Nkpolu-Oroworukwo, P.M.B. 5080, Port Harcourt, Rivers State, Nigeria

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The presence of tracheophyte-based vascular system of terrestrial plants had great impacts on the evolution of land plant biology. The vascular bundles have propelled the development of plants with increased stature, photosynthetic output and ability to colonize great expands of environmental habitats. A lot of progress has been made in an attempt to understanding the developmental programs involved in the formation and function of the vascular tissues.

Tracheophytes are distinguished from other plants by their highly developed vascular systems, made up of xylem and phloem, which facilitate the transport of water and nutrients to all parts of the plant, respectively[1]. This vascularization adaptation has allowed tracheophytes to become more fully terrestrial. Tracheophytes can be broken down into three classes; ferns, gymnosperms and angiosperms. Ferns are the least evolved of the tracheophytes; they have vascular systems and specialized leaf and root structures, but are still dependent on moist environments for reproduction.

Xylem is a complex tissue containing tracheary elements, fibers and parenchyma cells. Water is conducted by the tracheary elements, namely tracheids and/or vessel elements, which are dead cells with lignified and patterned secondary cell walls[2-4]. Parenchyma cells within the xylem store and/or secrete metabolites such as; starch, lignin monomers and resins[2].

The phloem tissue is also composed of multiple cell types, namely; sieve elements, companion cells, fibers and parenchyma cells. Large pores (called sieve pores) in the sieve plates conduct organic nutrients[5,6]. Phloem mother cells divide asymmetrically to give rise to sieve elements and companion cells, latter of which are rich in cytoplasm and organelles and contain a large nucleus[5,6]. Sieve elements and companion cells interact via plasmodesmata[5].

Extant land plant species have diverse types of Water Conducting Cells and Food Conducting Cells (WCCs and FCCs, respectively). In addition to exhibiting lignified WCCs, tracheophytes also possess a vascular bundle system, consisting of xylem containing tracheary elements and phloem containing sieve elements in close proximity. Although, the positional arrangements of xylem and phloem within vascular bundles vary by species, xylem and phloem basically occur as a set of vascular structures. This suggested that WCCs and FCCs share a common developmental program, at least partly, in tracheophytes. Indeed, xylem and phloem cells are generated from procambial and/or cambial cells[7-9].

The species used in this study to represent the three groups of tracheophytes were as follows: for Pteridophytes, *Nephrolepis exaltata* (L.) schott. (Sword Fern) was used, for Gymnosperm, *Cycas revoluta* Thunb. (Sago palm) *Pinus caribaea* Morelet (Caribbean Pine) and *Gnetum africanum* Welw. (African Jointfir) were used*,* while for the Angiosperm*, Commelina erecta* L. (Dayflower) (Monocotyledon) and *Talinum Triangulare* (Jacq.) Willd. (Waterleaf) (Dicotyledon) were used.

Research works on the comparative anatomy of all the groups of tracheophytes to show their evolutionary relationship is scanty. There is a dearth of information in this area. Therefore, this present study explores the valuable variations in the anatomical structures of the different groups of tracheophytes that can be used as an important taxonomic tool for the identification and differentiation of different species of the vascular plants.

MATERIALS AND METHODS

Study area: This research was carried out in the Department of Plant Science and Biotechnology of Rivers State University in Rivers state, Nigeria, from April, 2016 to February, 2018. Rivers state is one of the 36 states of Nigeria. According to census in 2006, the state has a population of 5,198,716, making it the 6th most populous state in the country.

Sources of plant materials: The materials for this study were collected from different parts of Rivers state, Nigeria, properly identified by using Flora of West Tropical Africa and deposited at the Rivers State University Herbarium, Nigeria.

Anatomical study: The plant materials for investigation were fixed in FAA solution (Formal dehyde: Acetic acid: alcohol in the ratio 1:1-18). After 24 h, the plant materials in the specimen bottles were removed and rinsed in three changes of distilled water in Petri dishes. The stems, leaves and rachis of plants were sectioned following the method of Agbagwa *et al.*[10]. Good sections were picked by using camel hair brush and dropped in the Petri dishes. Sections were lifted and placed on clean slides and excess water were removed using filter paper. One to two drops of 1% safranin was placed on the sections on the slides and filter paper used to remove excess stain.

The sections were mounted in 1-2 drops of glycerol and gently covered with cover slips to avoid trapping of air bubbles, then the slides were viewed by using BX22 light microscope. Photo micrographs were taken using Olympus DP70 digital camera.

Statistical analysis: The average (mean), standard deviation (STD) and range were determined using IBM SPSS Statistics 20.

RESULTS

The transverse sections taken from the middle area of the stems, rachis and leaves were observed. The anatomical features of all the plants studied are summarized in Table 1 and 2.

Anatomy of the Stem of *N. exaltata:* The anatomy of the stem of *N. exaltata* showed single layered epidermal cells with trichomes followed by hypodermis made of sclerenchyma cells. There were 5-6 layers of parenchyma cells followed by a layer of endodermal cell and pericycle completely surrounding the vascular bundles which were 3 in number, 1 large and 2 smaller ones. The vascular bundle is concentric and amphicribral that is, the phloem tissue completely surrounded the xylem tissue. The xylem tissue consists of just tracheids, which are primitive water conducting cells in the tracheophytes as shown in Table 1.

Anatomy of the stem of *C. revoluta*: The outermost layer of the rachis of *C. revoluta* is the single layered epidermis covered by a thick cuticle. Just beneath the epidermis is a hypodermis which is 2-3 layers in thickness on the adaxial side and many layered on the abaxial surface. The hypodermis is made of sclerenchymatous cells. The ground tissue which follows the hypodermis is made of parenchyma cells.

The most characteristic features of the rachis is the arrangement of vascular bundles in an omega-shaped outline. The vascular bundles vary in their structure at the base, centre and apex of the rachis. Each vascular bundle is surrounded by a single layered sclerenchymatous sheath. The vascular bundles are collateral and open. A single layered endodermis and 1-3 layered pericycle surrounds the vascular bundle. The xylem is mesarch. There are mucilaginous canals on the ground tissue which is completely filled with parenchyma cells as shown in Table 1.

Anatomy of the stem of *P. caribaea*: The anatomy of the young stem of *P. caribaea* showed the stem surrounded by a single layered cuticularized epidermis. Beneath the epidermis are multi-layered hypodermis consisting of lignified

Table 1: Summary of the anatomical structures of the stem and rachis of the plants studied

Character	*N. exaltata*	*C. revoluta*	*P. caribbaea*	*G. africanum*	*C. erecta*	*T. triangulare*
Epidermis	1 layer	1 layer	1 layer	1 layer	1 layer	1 layer
Trichomes	Present	Present	Absent	Absent	Absent	Absent
Hypodermis	Schlerenchy matous	Schlerenchy matous	Schlerenchy matous	Schlerenchy matous	Schlerenchy matous	Collenchymatous
Ground tissue	Parenchymatous	Same	Same	Same	Same	Same
Endodermis	1 layer	1 layer	Same	Same	Absent	Same
Pericycle	1 layer	1-3 layers	1 layer	1 layer	Absent	1-3 layers
Vascular bundles						
Type	Amphicribral	Collateral and open	Conjoint, collateral and open	Conjoint, collateral and open	Scattered and closed	Conjoint, collateral and open
Arrangement	Concentric	Omega shaped	In a ring	In a ring	Scattered	In a ring
Type of cells	Xylem tracheid and sieve cells in phloem	Tracheids and sieve cells	Tracheids and sieve tubes	Tracheids vessels and sieve tubes	Tracheids, vessels, sieve tubes and companion cells	Tracheids, vessels, sieve tubes and companion cells
No. of bundles	3	6	10	20-24	Numerous	Numerous
Bundle sheath	Absent	Absent	Absent	Absent	Present	Absent
Medullary rays	Absent	Absent	Present	Present	Absent	Present
Pith cavity	Present	Present	Present	Present	Absent	Present

Table 2: Summary of the anatomy and evolution of vascular bundles in the leaves and leaflets of the plants studied

Characters	*N. exaltata*	*C. revolusta*	*P. caribbaea*	*G. africanum*	*C. erecta*	*T. triangulare*
Epidermis	1 layer	1 layer	1 layer	1 layer	1 layer	1 layer
Mesophyll	Not Differentiated	Same	Same	Same	Same	Differentiated
Vascular bundles						
Type	Amphicribral	Collateral and mesarch	Conjoint, collateral and closed	Conjoint, collateral and endarch	Conjoint, collateral and closed	Conjoint, collateral and closed
Arrangement	At the center	Mesarch	Conjoint	Anarch	Parallel	Anarch
Number	1	1	2	3	4	4
Cambium	Absent	Absent	Absent	Present	Absent	Absent
Bundle sheath	Absent	Sclerenchyma	Absent	Absent	Chlorenchyma	Parenchyma

sclerenchymatous cells. Beneath the hypodermis are thin walled parenchyma cells containing chloroplasts and resin canals. The endodermis and pericycle are not visible. The vascular bundles are conjoint, collateral and open arranged in a ring. The bundles are separated from each other by narrow medullary rays as shown in Table 1.

Anatomy of the stem of *G. africanum*: The anatomy of *G. africanum* showed a single layered epidermis. Cuticle is thick and the ground tissue is multi-layered made of sclerenchymas cells. Endodermis and pericycle are inconspicuous. There is a ring of 20-24 conjoint, collaleral and endarch vascular bundles separated from one another by medullary rays. Xylem tissue is composed of a large number of tracheids and few vessels. The phloem is made of sieve cells and phloem parenchyma. The pith cavity is filled with parenchyma cells as shown in Table 1.

Anatomy of the stem of *C. erecta*: The transverse section of the stem of *C. erecta,* a monocotyledonous plant showed the epidermis which is single layered with closely packed cells, covered by a thick cuticle. Beneath the epidermis are 1-2 layers of thick-walled sclerenchyma cells followed by thin-walled parenchyma cells which made up the ground tissue. Endodermis, pericycle and pith cavity are absent. The vascular bundles are scattered throughout the ground tissue. The bundles nearer the rind of the stem are smaller and closer to one another. The vascular bundles are closed. Each vascular bundle is surrounded by sclerenchymatous sheath as shown in Table 1.

Anatomy of the stem of *T. triangulare*: The anatomy of the stem of *T. triangulare* showed a single layered epidermal cells covered by a thick cuticle. Beneath the epidermis are 2-3 layers of collenchymas cells followed by a few layers of parenchyma cells. A single layered endodermis followed by multi-layered pericycle surrounded the vascular bundles. The vascular bundles were arranged in a ring, though some were scattered in the pith region. The vascular bundles are conjoint, collateral and open. The pith cavity is filled with parenchyma cells as shown in Table 1.

Anatomy of the leaflet of *N. exaltata*: The transverse section of the leaflet of *N. exaltata* showed a layer of epidermis covered by the cuticle. Beneath the epidermis are the hypodermis and parenchyma cells followed by a single vascular bundle which is amphicribral in nature. There is a notch at the adaxial surface of the leaflet as shown in Table 2.

Anatomy of the leaflet of *C. revoluta*: The anatomy of the leaflet of *C. revoluta* showed strong xerophytic features. There are thick-walled epidermal cells with very thick cuticle. Beneath the epidermis is a layer of hypodermis which is several cells thick in the middle region. The mesophyll is differentiated into palisade and spongy mesophyll. The vascular bundle is mesarch. Cambium is absent. A sclerenchymatous sheath surrounded the single vascular bundle. Mucilaginous canals are present as shown in Table 2.

Anatomy of the leaflet of *P. caribaea*: Anatomical features of *P. caribaea* showed epidermal cells extremely thick-walled and cuticularized. Beneath the epidermis is hypodermis made of one-two layers of sclerenchyma cells. There are air spaces beneath each stomata. Mesophyll is not differentiated into palisade and spongy tissues. There is a layer of endodermis with multi layered pericycle. There are 2 vascular bundles in between them of which are transfusion tissue. Each bundle is conjoint, collateral and open as shown in Table 2.

Anatomy of the leaf of *G. africanum*: The anatomy of the leaf of *G. africanum* showed undulated upper and lower single layered epidermal cells covered by a cuticle. The mesophyll is differentiated into palisade and spongy tissue vascular bundles in the midrib are arranged in the form of an arch followed by patches of stone cells on the lower side. Bundles are conjoint, collateral and endarch as shown in Table 2.

Anatomy of the leaf of *C. erecta*: Transverse section of the leaves of *C. erecta* showed a layer of epidermis covered by thick cuticle. The mesophyll is not differentiated. There are a few large, bulliform cells in the upper epidermis. There are 4 vascular bundles, arranged in parallel serves, conjoint, collateral and closed. Each vascular bundle is surrounded by chlorenchymatous bundle sheath as shown in Table 2.

Anatomy of the leaf of *T. triangulare*: Anatomy of *T. triangulare* leaf showed a single layered epidermis covered by a thick cuticle both upper and lower epidermis. The mesophyll is differentiated into palisade and spongy cells. Vascular bundles are conjoint, collateral and closed, covered by a bundle sheath of parenchyma cells as shown in Table 2.

DISCUSSION

Tracheophytes are group of plants distinguished from other plants by their highly developed vascular systems, which enables the transportation of water and mineral salts and prepared food to all parts of the plant body. This vascularization has enabled plants to adapt to land.

In this study, the anatomy and vascular tissues in the stem, rachis, leaf and leaflets of all the groups of tracheophytes were studied. The ferns, pteridophytes are the first group of tracheophytes represented in this study by *N. exaltata* followed by the second group, the gymnosperms, grouped into Cycadaceae, Ginkgoaceae, Gnetaceae and Coniferaceae. They were represented by *C. revoluta, G. africanum* and *P. caribaea*, respectively. The third group of tracheophytes is the angiosperms which are grouped into monocotyledons and dicotyledons, here represented by *C. erecta* and *T. traingulare*, respectively.

The stem and rachis anatomy showed evolution of vascular bundles in the tracheophytes: Amphicribral vascular bundle in the ferns which is a more primitive type of bundle, made up of tracheids and 3 in number. The cycad plant consist of collateral and open vascular bundles which indicated that the xylem and phloem tissues are joined together, but separated by the cambium which enabled the plant to undergo secondary growth. The stem of pinus was similar to that of *C. revoluta* only that the vascular bundles were conjoint, that is, arranged side by side in a ring and higher in number. The vascular bundles in *G. africanum* were more advanced and similar to the bundles in angiosperms since, it contained xylem vessels which are only found in angiosperms, a more advanced group of tracheophytes and coincided with the findings by Esau[11] and Pandey[12]. The vessel element conducts water more than the tracheids. The gnetum was followed by *C. erecta* which has scattered and more numerous bundles, but lacking cambium and as a result; they do not undergo secondary growth.

The last group of tracheophytes, the angiosperms contains cambium in addition to xylem and phloem and the bundles were arranged in a ring and numerous in number in *T. triangulare*, the dicotyledon. The anatomical properties of these plants have the general features of dicotyledons[11,13,14]. This has enabled these plants to survive more on land than other groups of tracheophytes.

Variations in number and arrangement of vascular bundles in anatomical structures have been used to show variations among species[11,13,15,16]. For instance, in their studies, Ekeke and Mensah[15] noted that the number of vascular traces in the midrib of members of Asteraceae varied from one species to another and is diagnostic. Also, Agbagwa and Ndukwu[16] reported that the number of vascular bundles in the petioles of members of Cucurbita could be used to distinguish them.

In this study, the number of vascular bundles in the stems, rachis, leaf and leaflets varied from one group to another (Table 1 and 2). This variation in the number of vascular bundles is diagnostic and could be used to distinguish the different groups of vascular plants. This findings, therefore, supported the variation in number of vascular bundles in plant anatomical structures are diagnostic[13,15,16] and indicated the evolution of vascular bundles from the simplest to the most complicated group which are the angiosperms. The results of the study shall enhance the establishment of the taxonomic status of the species in this group. Further studies should be carried out on the anatomy of the organs of other members of these groups of tracheophytes to ascertain their evolutionary relationship.

CONCLUSION

This study has revealed the evolution of vascular bundles among the tracheophytes. It showed different level of evolutionary development among the plants with the first group of plants, the ferns, being the least developed and the evolutionary relationship among the plants. These are useful taxonomic characters for delimiting the species especially when combined with the existing data on the species.

SIGNIFICANCE STATEMENT

This study discovered variation in the number of vascular bundles from the simplest plant group to the most complex, that is, from the fern to the angiosperm which is the most complex plant group. This is useful for easy identification of these plants. The similarities among these group of plants point towards the same evolutionary origin while the differences indicated the different level of evolutionary development among the plants. This will help the researchers to uncover the critical areas of plant evolution that many researchers were not able to explore.

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