TAXONOMICIMPLEMENTATIONOFROOTANATOMYINIDENTIFICATION AND DIFFERENTIATION OF SOME RUTACEAE TAXA

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Abstract

The Rutaceae family is a large group of flowering plants placed in the order Sapindales. This study involved an anatomical study of transverse sections of roots from selected species within the Rutaceae family. Light and scanning electron microscope was used for anatomical study. Different anatomical characteristics exodermis length and width, cork cells length and width, cortex parenchyma cells length and width, endodermis length, phloem strand length, xylem tracheae diameter, vessel length and width and pith cells diameter were examined. The exodermis cells length ranges from 47-145 μ m. The exodermis cells width ranges from 24-78.5 μ m. Cork Cells Length ranged between 46-120.5 μ m. The maximum endodermis length observed was almost 110-120.5 μ m in *Citrus Sinensis*. The maximum phloem strand length observed in *Citrus Paradisi* was 175-182 μ m. Maximum pith diameter was seen in *Citrus Grandis*. In conclusion, the characteristics observed in the transverse sections of roots showed significant variations, enabling the differentiation of plants within the Rutaceae family. This is the first documented study on root section cutting in species of the Rutaceae Taxa.

Keywords: Rutaceae, Light and Scanning Electron Microscope, Endodermis Length, Phloem Strand, Exodermis, Pith Diameter.

INTRODUCTION

The Rutaceae family is a diverse group of flowering plants that includes many economically and ecologically important species. (Mabberley, 2018). The root anatomy of plants within the Rutaceae family can exhibit variations, but there are some general characteristics that are commonly observed such as fibrous root systems, root hairs, secondary growth and mycorrhizal associations. (Ulukus *et al.*, 2016).

Rutaceae plants typically have a fibrous root system, which means that they possess a dense network of thin roots emerging from the base of the stem. This type of root system helps in anchoring the plant firmly in the soil and facilitates efficient absorption of water and nutrients. (Latiff *et al.*, 2019).

The roots of Rutaceae plants often develop root hairs. Root hairs are tiny, elongated structures that emerge from the root surface and play a crucial role in increasing the surface area for water and nutrient absorption. (Barkatullah *et al.*, 2014).

In certain Rutaceae species, roots may serve as storage organs for water, nutrients, or even secondary metabolites. These specialized roots may be modified for storage purposes and can be important for the survival of the plant in challenging environmental conditions. Some Rutaceae species may develop adventitious roots, which are roots that form from non-root tissues, such as stems or leaves. Adventitious roots can aid in the plant's adaptation to various environmental conditions. (Ezeabara and Okeke, 2016).

Rutaceae plants may form symbiotic relationships with mycorrhizal fungi. Mycorrhizae enhance nutrient absorption by increasing the root's effective surface area and facilitating the uptake of minerals, especially phosphorus. Some members of the Rutaceae family exhibit secondary growth in their roots. Secondary growth involves the formation of secondary tissues, such as vascular tissues (xylem and phloem), which contribute to the thickening of the root over time.

This is more commonly seen in woody species within the family. It's important to note that there can be considerable variability in root anatomy among different genera and species within the Rutaceae family. (Thorne, 2012).

Additionally, factors such as environmental conditions and the plant's life stage can influence root development and structure. Studying the root anatomy of Rutaceae plants is essential for understanding their ecological roles, adaptation strategies, and potential uses in various applications, including horticulture and medicine.

(Waterman, 2015). It's important to note that the specific features observed in a transverse section may vary among different species within the Rutaceae family. Additionally, the presence of secondary growth, specialized cells, and other adaptations can contribute to the diversity of root anatomy within this plant family.

MATERIALS AND METHODS

For anatomical investigations, fresh plant materials fixed in a mixture of Formalin-Acetic acid, glacial acetic acid and 70 percent ethanol were used for preparing cross-sections of roots. The manual process was utilized to obtain these cross-sections through commercial razor blades, these cross-sections were subsequently stained with either safranin or acetyl blue following the methods outlined by Paiva *et al.*, (2016).

The stained cross-sections underwent dehydration through an ethanol gradient and xylol (99.5%) before being mounted on microscopic slides using canada balsam. Examination of the slides was conducted using both light and scanning electron microscopes. The analysis of root characteristics adhered to the methodology proposed by Graciano-Ribeiro *et al.*, (2006), incorporating some novel traits. Anatomical data description followed the terminology of Tomlinson *et al.* (2011).

Key quantitative features investigated included the number of layers, shapes, and thickness of epidermal cells, the number of cortex layers and their thickness, the number and length of air spaces, vascular bundle diameter and the number of xylary arches. Additionally, observations encompassed the number of metaxylem arches, inner diameter of wide metaxylem arch, maximum length of phloem strand and pith diameter."

RESULTS

In the cross-sectional analysis of the root, various significant cells were identified, including periderm, cortex parenchyma cells, endodermis, xylary elements, xylem arches, vessels, tracheids, phloem strands, pith rays and root hairs. Secondary growth, characteristic of a dicotyledonous plant, was evident in the root. The outermost layer of the root featured a periderm in the form of an epidermis, with varying thickness.

1. Citrus reticulata Blanco.

Root Anatomy The transverse section of the root was circular in outline having outer layer called exodermis followed by the cork cells. The exodermis is smooth with thin walls, composed of mostly a single layer and their length ranged from 47-50µm and width observed was 24-29.5µm.

The cork cells were composed of many layers having a length of 46-50.5µm and the width of the cork cells ranged from 24.5-28µm. Under the cork cells cortex parenchyma cells are present. Almost 5-9 cortex parenchyma cells layers were present. The cortex parenchyma cells were of different shapes and sizes. Mostly tangentially elongated shaped parenchyma cells were seen.

The length of the parenchyma cells ranged from 45-51.5µm and their width ranged from 20-23µm. Narrow inter-cellular spaces containing numerous calcium oxalate prisms and oil glands were also seen among parenchyma cells.

Endodermis observed was single layered and mostly of circular-polygonal shaped cells. Endodermal cells varied in length and were ranged from 40-44.5 μ m. The endodermis is indistinct and is followed by parenchymatous pericycle having patches of pericyclic fibres. The pericyclic fibres are surrounded by distinct crystal sheath. The vascular cylinder diameter observed was almost 130-149 μ m. The maximum length of the phloem strand observed was 65-69 μ m.

The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was 9-13 μ m. The vessels are porous and are mostly circular in shape. The size of the vessels varied. The maximum length of vessels seen was 45-87 μ m and the width of the vessels ranged between 52-120 μ m. The diameter of the pith cells observed was 21-43 μ m. The pith rays were comprised of 1-2 rowed circular to polygonal shaped cells. Root hairs were also seen.



a. LM showing xylem vessels and pericycle fibers



b. SEM showing the xylem vessels

Fig 1: Citrus reticulata Blanco

2. Citrus limon (Linn.) Burm.f.

Root Anatomy The circular outline of the transverse section was consisted of an outer layer, the exodermis which was followed by the layers of cork cells. The exodemis observed was smooth with thick walls, composed of many layers, mostly 6-9 layered, and their length ranged from 50.5-56.5µm and width observed was 27-31µm. The cork cells were composed of many layers having a length of 35-37.5µm and the width of the cork cells ranged from 17.5-21µm. Under the cork cells cortex parenchyma cells were seen. Almost 9-11 cortex parenchyma cells layers were seen. The shapes and sizes of the cortex parenchyma cells were different. Mostly vasicentric aliform parenchyma cells were seen. The length of the parenchyma cells ranged from 37-44µm and their width ranged from 21.5-29µm. Narrow inter-cellular spaces containing numerous calcium oxalate prisms and oil glands were also seen among parenchyma cells. Endodermis observed was single to many layered layered and mostly of circular-polygonal shaped cells. Endodermal cells varied in length and were ranged from 55-61µm. The endodermis seen aws indistinct and was followed by the parenchymatous pericycle having pericyclic fibres. The pericyclic fibres were surrounded by the distinct crystal sheath. The vascular cylinder

diameter observed was almost 145-169 μ m. The maximum length of the phloem strand observed was 89-99 μ m. The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae seen was in the range of 12-17 μ m. The vessels observed were porous and were mostly circular in shape. The size of the vessels varied. The length of vessels seen was ranged between 55-69.5 μ m and the width of the vessels ranged between 42-47.5 μ m. The diameter of the pith cells observed was 30-47.5 μ m. The pith rays were comprised of 1-2 rowed circular shaped cells. Root hairs were also seen.



a. Showing multilayered exodermis with thick walls and xylem vessels



b. SEM showing cortex parenchyma cells

Fig 2: Citrus limon (Linn.) Burm.f.

3. Citrus sinensis Linn. Osbeck

Root Anatomy The outer layer exodemis was followed by the cork cells. The exodermis cells were smooth with thin walls, composed of mostly a single layer and their length ranged from 70-83.5µm and width observed was 45-47.5µm. The cork cells were composed of many layers having a length of 47-54µm and the width of the cork cells ranged from 27.5-32 µm. Under the cork cells cortex parenchyma cells are present. Almost 13-15 cortex parenchyma cells layers were present. The cortex parenchyma cells were of different shapes and sizes. Mostly irregular to polygonal shaped parenchyma cells were seen. The length of the parenchyma cells ranged from 40-53.5µm. Narrow inter-cellular spaces containing numerous calcium oxalate prisms and oil glands were also seen among parenchyma cells. Endodermis

observed was single-many layered and mostly of irregular-polygonal shaped cells. Endodermal cells varied in length and were ranged from 110-120.5 μ m. The endodermis is indistinct and is followed by parenchymatous pericycle having patches of pericyclic fibres. The pericyclic fibres are surrounded by distinct crystal sheath. The vascular cylinder diameter observed was almost 170-179 μ m. The maximum length of the phloem strand observed was 105-129 μ m. The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was 26-33 μ m. The vessels are porous and are mostly circular-oval in shape. The size of the vessels varied. The maximum length of vessels seen was 115-127 μ m and the width of the vessels ranged between 75-80.5 μ m. The diameter of the pith cells observed was 51-60 μ m. The pith rays were comprised of 1-2 rowed mostly polygonal shaped cells. Root hairs were also seen.



a. Showing single layered exodermis with cortex parenchyma and xylem and phloem



b. SEM showing polygonal shaped cells

Fig 3: Citrus sinensis Linn. Osbeck

4. Citrus medica var acida Brandis.

Root Anatomy The outer layer seen was exodemis which was followed by the cork cells. The exodermis cells seen were with smooth and thick walls, composed of mostly one to many layers, mostly 6-9 layered and their length ranged from 70-90 μ m and their width was ranged between 30-36.5 μ m. The cork cells were composed of many layers having a length of 47-51 μ m and the width of the cork cells ranged from 30.5-404 μ m. Under the cork cells cortex parenchyma cells were observed. Almost 23-29 cortex parenchyma cells

layers were seen. The cortex parenchyma cells were of different shapes and sizes. Mostly polygonal shaped parenchyma cells were observed. The length of the parenchyma cells ranged from 75-86.5µm and their width ranged from 54-63µm. Narrow inter-cellular spaces containing numerous calcium oxalate prisms and oil glands were also seen among parenchyma cells. Endodermis observed was single-many layered and mostly of polygonal shaped cells. Endodermal cells varied in length and were ranged from 90-110µm. The endodermis is indistinct and is followed by parenchymatous pericycle having pericyclic fibres. The pericyclic fibres are surrounded by distinct crystal sheath. The vascular cylinder diameter observed was almost 180-199.5µm. The maximum length of the phloem strand observed was 109-118.5µm. The xylem is consisted of tracheids. medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was 66-73.5µm. The vessels are porous and are mostly circular-oval in shape. The size of the vessels varied. The maximum length of vessels seen was 145-166.5µm and the width of the vessels ranged between 112-119.5µm. The diameter of the pith cells observed was 91-106.5 µm. The pith rays were comprised of 1-2 rowed mostly polygonal shaped cells. Root hairs were also seen.



a. LM showing smooth xylem vessels



b. Polygonal shaped parenchyma cells Fig 4: *Citrus medica var acida Brandis*

5. Citrus limetta Risso.

Root Anatomy

The exodemis was followed by the layers of cork cells. The exodermis cells seen were with smooth and thick walls, composed of mostly a single layer and their length ranged from 107-119.5µm and their width was ranged between 73-78.5µm. The cork cells were composed of many layers having a length of 92-120.5µm and the width of the cork cells ranged from 67-74.5µm. Under the cork cells cortex parenchyma cells were observed. Almost 15-22 cortex parenchyma cells layers were seen. The cortex parenchyma cells were of different shapes and sizes.

Mostly irregular shaped parenchyma cells were observed. The length of the parenchyma cells ranged from 105.5-116.5µm and their width ranged from 94-103µm. Narrow intercellular spaces containing numerous calcium oxalate prisms and oil glands were also seen among parenchyma cells. Endodermis observed was single-many layered and mostly of irregular-polygonal shaped cells. Endodermal cells varied in length and were ranged from 89-103µm. The endodermis is indistinct and is followed by parenchymatous pericycle having patches of pericyclic fibres.

The pericyclic fibres are surrounded by distinct crystal sheath. The vascular cylinder diameter observed was almost 170-176µm. The maximum length of the phloem strand observed was 91-110µm. The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was 104.5-111.5µm. The vessels are porous and are mostly circular-oval in shape.

The size of the vessels varied. The maximum length of vessels seen was $152.5-170\mu$ m and the width of the vessels ranged between $110-119.5\mu$ m. The diameter of the pith cells observed was $101-118\mu$ m. The pith rays were comprised of 1-2 rowed mostly polygonal shaped cells. Root hairs were also seen.



a. LM showing smooth thick walled exodermis and root hairs



b. SEM showing exodermis, medullary rays and xylem vessels.

Fig 5: Mentha haplocalyx L

6. Citrus grandis Osbeck.

Root Anatomy

The exodermis cells observed were with smooth and thick walls, composed of one to many layers made up of suberised cells, almost 10-14 layered and their length ranged from 77.5-81.5.5 μ m and their width was ranged between 45.5-56.5 μ m. The cork cells were composed of many layers having a length of 102-110 μ m and the width of the cork cells ranged from 80.5-86 μ m. Under the cork cells cortex parenchyma cells were observed.

Almost 17-23 cortex parenchyma cells layers were seen. The cortex parenchyma cells were of different shapes and sizes. Mostly irregular shaped parenchyma cells were observed. The length of the parenchyma cells ranged from 90-100µm and their width ranged from 67-73.5µm. Narrow inter-cellular spaces containing numerous calcium oxalate prisms and oil glands were also seen among parenchyma cells. The endodermis was not observed and detected in the transverse section of this species.

A collateral arrangement of the vascular bundles was seen in the transverse section and the vascular cylinder diameter observed was almost $161-176\mu m$. The length of the phloem strand was ranged between $87.5-90\mu m$. The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was $100.5-105\mu m$.

The vessels are porous and are mostly circular-oval in shape. The size of the vessels varied. The maximum length of vessels seen was $99.5-110.5\mu$ m and the width of the vessels ranged between $70-78.5\mu$ m. The diameter of the pith cells observed was $91.5-108.5\mu$ m. The pith rays were comprised of 1-2 rowed mostly polygonal shaped cells. Root hairs were also seen.



a. LM showing thick walled exodermis, cortex parenchyma, trachea and medullaery rays



b. SEM of root of *C. grandis* Fig 6: *Citrus grandis* Osbeck

7. Citrus aurantium Linn.

Root Anatomy

The circular outline of the transverse section was consisted of an outer layer, the exodermis which was followed by the layers of cork cells. The exodemis observed was smooth with thick walls, composed of many layers, mostly upto 7 layers and their length ranged from $51.5-55.5\mu$ m and width observed was $32.5-39\mu$ m. The cork cells were rectangular in shape with suberised walls and were composed of many layers having a length of 93-99 μ m and the width of the cork cells ranged from 76-80.5 μ m. Under the cork cells cortex parenchyma cells were seen. Almost 16-20 cortex parenchyma cells layers were seen. The shapes and sizes of the cortex parenchyma cells were different. Mostly polygonal shaped parenchyma cells were seen with large amount of starch grains. The length of the parenchyma cells ranged from 59.5-64 μ m and their width ranged from 44.5-60 μ m. Large amount of sclereids in the form of patches were also seen in the cortex

parenchyma cells. The sclereids were polygonal, oval and rounded in shape. Endodermis observed was many layered and mostly of circular-polygonal shaped cells. Endodermal cells varied in length and were ranged from 95-101 μ m. The vascular cylinder diameter observed was almost 145-150 μ m. A narrow ring of phloem with few layers of parenchyma cells was also seen. The maximum length of the phloem strand observed was 60-69 μ m. The xylem occurred as a central core and occupied majority of the portion and was consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae seen was in the range of 22.5-27.5 μ m. The vessels observed were porous and were mostly circular in shape. The size of the vessels varied. The length of vessels seen was ranged between 75-80 μ m and the width of the vessels ranged between 52-67 μ m. The diameter of the pith cells observed was 39-45 μ m. The pith rays were comprised of 1-2 rowed circular shaped cells. Root hairs were also seen



a. LM showing cork cells, cortex parenchyma, phloem and xylem vessels



b. SEM showing root hairs and exodermis Fig 7: *Citrus aurantium* Linn

8. Citrus aurantifolia Christm

Root Anatomy

The exodemis is smooth with thick walls, composed of mostly many layers, almost 5-7 layers were observed and their length ranged from 107.5-115 µm and width observed was 47-55.5µm. The cork cells were composed of many layers having a length of 86-92.5µm and the width of the cork cells ranged from 63-66µm. Under the cork cells cortex parenchyma cells are present. Almost 9-13 cortex parenchyma cells layers were present. The cortex parenchyma cells were of different shapes and sizes. Mostly irregularpolygonal shaped parenchyma cells were seen. The length of the parenchyma cells ranged from 30.5-35µm and their width ranged from 19-21.5µm. Narrow inter-cellular spaces were also seen among parenchyma cells. Endodermis observed was many layered, mostly 5-8 layers and mostly of circular-semicircular and polygonal shaped cells. Endodermal cells varied in length and were ranged from 60-64µm. The endodermis is followed by parenchymatous pericycle having pericyclic fibres. The pericyclic fibres are surrounded by distinct crystal sheath. The vascular cylinder diameter observed was almost 170-179µm. The maximum length of the phloem strand observed was 115-120µm. The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was 15-18µm. The vessels are porous and are mostly circular in shape. The size of the vessels varied. The maximum length of vessels seen was 43-47.5µm and the width of the vessels ranged between 40-45.5µm. The diameter of the pith cells observed was 23.5-45 µm. The pith rays were comprised of 1-2 rowed circular to polygonal shaped cells. Root hairs were also seen.



a. LM showing thick endodermis and vascular bundles



b. SEM showing cortex parenchyma and phloem

Fig 8: Citrus aurantifolia Christm

9. Citrus paradisi Macfadyen

Root Anatomy The exodemis is smooth with thick walls, composed of mostly many layers, almost 6-9 layers and their length ranged from 87-90 μ m and width observed was 53-59 μ m. The cork cells were composed of many layers having a length of 77.5-82 μ m and the width of the cork cells ranged from 43-47.5 μ m. Under the cork cells cortex parenchyma cells were present.

Almost 15-17 cortex parenchyma cells layers were present. The cortex parenchyma cells were of different shapes and sizes. Mostly polygonal shaped parenchyma cells were seen. The length of the parenchyma cells ranged from 100.5-105µm and their width ranged from 79.5-85µm. Narrow inter-cellular spaces were also seen among parenchyma cells. Endodermis observed was mostly 4-5 layered and mostly of circular-polygonal shaped cells. Endodermal cells varied in length and were ranged from 81-94µm.

The endodermis is followed by parenchymatous pericycle having pericyclic fibres. The pericyclic fibres are surrounded by crystal sheath. The vascular cylinder diameter observed was almost 200-209 μ m. The maximum length of the phloem strand observed was 175-182 μ m. The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was 29-37 μ m.

The vessels are porous and are mostly circular in shape. The size of the vessels varied. The maximum length of vessels seen was 52-60.5 μ m and the width of the vessels ranged between 39-44 μ m. The diameter of the pith cells observed was 30.5-37.5 μ m. The pith rays were comprised of 1-2 rowed circular to polygonal shaped cells. Root hairs were also seen.



a. LM of root showing cortex parenchyma, vascular bundles and medullary rays



b. SEM showing polygonal shaped cells

Fig 9: Citrus paradisi Macfadyen

10. Murraya koenigi Linn.

Root Anatomy The exodemis is smooth with thick walls, composed of mostly single layer and their length ranged from 83-85 μ m and width observed was 37.5-40 μ m. The cork cells were composed of many layers having a length of 62-66.5 μ m and the width of the cork cells ranged from 28-32 μ m. Under the cork cells cortex parenchyma cells were present. Almost 10-13 cortex parenchyma cells layers were present.

The cortex parenchyma cells were of different shapes and sizes. Mostly irregular and polygonal shaped parenchyma cells were seen. The length of the parenchyma cells ranged from 78.5-84µm and their width ranged from 68-74.5µm. Narrow inter-cellular spaces were also seen among parenchyma cells. Endodermis observed was mostly 5-7 layered and mostly of irregular-polygonal shaped cells. Endodermal cells varied in length and were ranged from 65-69µm.

The endodermis is followed by parenchymatous pericycle having pericyclic fibres. The pericyclic fibres are surrounded by crystal sheath. The vascular cylinder diameter observed was almost $70-77\mu m$. The maximum length of the phloem strand observed was

87-90.5μm. The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was 31-35.5μm. The vessels are porous and are mostly circular in shape.

The size of the vessels varied. The maximum length of vessels seen was $41.5-46.5\mu$ m and the width of the vessels ranged between $30.5-34.5\mu$ m. The diameter of the pith cells observed was 27-30.5 μ m. The pith rays were comprised of 1-2 rowed circular to polygonal shaped cells. Root hairs were also seen.



a. LM of root showing exodermis, phloem and xylem vessels



c. SEM of root xylem vessels Fig 10: *Murraya koenigi* Linn

11. Murraya paniculata Linn.

Root Anatomy The exodemis is smooth with thick walls, composed of mostly one layer and their length ranged from 77-80µm and width observed was 49.5-52.5µm. The cork cells were composed of many layers having a length of 47.5-53µm and the width of the cork cells ranged from 24-28.5µm. Under the cork cells cortex parenchyma cells were present. Almost 15-18 cortex parenchyma cells layers were present. The cortex parenchyma cells were of different shapes and sizes. Mostly circular, semi-circular and polygonal shaped parenchyma cells were seen. The length of the parenchyma cells ranged from 62.5-68µm and their width ranged from 62.5-68µm.

spaces were also seen among parenchyma cells. Endodermis observed was mostly 13-15 layered and mostly of polygonal shaped cells. Endodermal cells varied in length and were ranged from 47.5-51.5µm. The endodermis is followed by parenchymatous pericycle having pericyclic fibres. The pericyclic fibres are surrounded by crystal sheath. The vascular cylinder diameter observed was almost 52.5-58µm. The maximum length of the phloem strand observed was 66.5-70µm. The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was 22.5-27µm. The vessels are porous and are mostly circular in shape. The size of the vessels varied. The maximum length of vessels seen was 36-40µm and the width of the vessels ranged between 22-26µm. The diameter of the pith cells observed was 30-33 µm. The pith rays were comprised of 1-2 rowed circular to polygonal shaped cells. Root hairs were also seen.



a. LM of root showing xylem vessels, phloem and pericycle fibers



b. SEM showing medullary rays and vascular bundles

Fig 11: Murraya paniculata Linn

12. Zanthoxylum armatum DC.

Root Anatomy The exodemis is smooth with thin walls, composed of mostly many layers, almost 12-16 layers and their length ranged from 99-103.5µm and width observed was 40.5-46µm. The cork cells were composed of many layers having a length of 49-54.5µm and the width of the cork cells ranged from 30-35.5µm. Under the cork cells cortex parenchyma cells were present. Almost 21-24 cortex parenchyma cells layers were present. The cortex parenchyma cells were of different shapes and sizes. Mostly circular to polygonal shaped parenchyma cells were seen. The length of the parenchyma cells

ranged from 50-57µm and their width ranged from 33.5-39µm. Narrow inter-cellular spaces were also seen among parenchyma cells. Endodermis observed was mostly single layered and mostly of polygonal shaped cells. Endodermal cells varied in length and were ranged from 57.5-61µm. The endodermis is followed by parenchymatous pericycle having pericyclic fibres. The pericyclic fibres are surrounded by crystal sheath. The vascular cylinder diameter observed was almost 66-70µm. The maximum length of the phloem strand observed was 77-80.5µm. The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was 31-39µm. The vessels are porous and are mostly circular in shape. The size of the vessels varied. The maximum length of vessels seen was 42-44.5µm and the width of the vessels ranged between 30-37.5µm. The diameter of the pith cells observed was 45-47µm. The pith rays were comprised of 1-2 rowed circular to polygonal shaped cells. Root hairs were also seen.



a. LM root showing xylem vessels and fibers



b. SEM of TS of root of Zanthoxylum armatum

Fig 12: Zanthoxylum armatum DC

13. Aegle marmelos Linn.

Root Anatomy The exodemis is smooth with thin walls, composed of mostly many layers, almost 6-9 layers and their length ranged from 91-95 μ m and width observed was 52.5-56.5 μ m. The cork cells were composed of many layers having a length of 60-68 μ m and the width of the cork cells ranged from 44.5-48 μ m. Under the cork cells cortex

parenchyma cells were present. Almost 17-20 cortex parenchyma cells layers were present. The cortex parenchyma cells were of different shapes and sizes. Mostly circular to irregular shaped parenchyma cells were seen. The length of the parenchyma cells ranged from 93.5-97.5µm and their width ranged from 42-49.5µm. Narrow inter-cellular spaces were also seen among parenchyma cells. Endodermis observed was mostly single layered and mostly of irregular shaped cells. Endodermal cells varied in length and were ranged from 88.5-92.5µm. The endodermis is followed by parenchymatous pericycle having pericyclic fibres. The pericyclic fibres are surrounded by crystal sheath. The vascular cylinder diameter observed was almost 86-90µm. The maximum length of the phloem strand observed was 117.5-124µm. The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was 53-59µm. The vessels are porous and are mostly circular in shape. The size of the vessels varied. The maximum length of vessels seen was 55-60.5µm and the width of the vessels ranged between 40.5-47.5µm. The diameter of the pith cells observed was 56-63µm. The pith rays were comprised of 1-2 rowed circular to polygonal shaped cells. Root hairs were also seen.



a. LM root showing xylem vessels, stele and xylary fibers



b. SEM showing xylem vessels

Fig 13: Aegle marmelos Linn

14. Fortunella japonica

Root Anatomy The exodemis is smooth with thick walls, mostly rectangular shaped cells, composed of many layers, almost 4-5 layers and their length ranged from 137-145µm and width observed was 77.5-87µm. The cork cells were composed of many layers having

a length of 98-104µm and the width of the cork cells ranged from 69.5-74µm. Under the cork cells cortex parenchyma cells were present. Almost 19-23 cortex parenchyma cells layers were present. The cortex parenchyma cells were of different shapes and sizes. Mostly polygonal and irregular shaped parenchyma cells were seen. The length of the parenchyma cells ranged from 143-151.5µm and their width ranged from 72-79µm. Narrow inter-cellular spaces were also seen among parenchyma cells. Endodermis observed was mostly 1-4 layered and mostly of irregular shaped cells. Endodermal cells varied in length and were ranged from 108-115µm. The endodermis is followed by parenchymatous pericycle having pericyclic fibres. The pericyclic fibres are surrounded by crystal sheath. The vascular cylinder diameter observed was almost 106-110µm. The maximum length of the phloem strand observed was 144.5-150.5µm. The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was 77-84µm. The vessels are porous and are mostly circular in shape. The size of the vessels varied. The maximum length of vessels seen was 79.5-84µm and the width of the vessels ranged between 62.5-70µm. The diameter of the pith cells observed was 66.5-75.5µm. The pith rays were comprised of 1-2 rowed circular to polygonal shaped cells. Root hairs were also seen.



a. LM root showing exodermis and xylem vessels



b. SEM showing endodermis and medullary rays

Fig 14: Fortunella japonica

15. Skimmia laureola DC.

Root Anatomy The exodemis is smooth with thick walls, composed of many layers, almost 8-10 layers and their length ranged from 122.5-126µm and width observed was

69-72µm. The cork cells were composed of many layers having a length of 97-102µm and the width of the cork cells ranged from 72-76.5µm. Under the cork cells cortex parenchyma cells were present. Almost 20-26 cortex parenchyma cells layers were present. The cortex parenchyma cells were of different shapes and sizes. Mostly polygonal shaped parenchyma cells were seen. The length of the parenchyma cells ranged from 130.5-137µm and their width ranged from 66-69.5µm. Narrow inter-cellular spaces were also seen among parenchyma cells. Endodermis observed was mostly single layered and mostly of polygonal shaped cells. Endodermal cells varied in length and were ranged from 101.5-107.5µm. The endodermis is followed by parenchymatous pericycle having pericyclic fibres. The pericyclic fibres are surrounded by crystal sheath. The vascular cylinder diameter observed was almost 112.5-120µm. The maximum length of the phloem strand observed was 160-165µm. The xylem is consisted of tracheids, medullary rays, crytal sheaths and xylem vessels. The diameter of tracheae observed was 90.5-94µm. The vessels are porous and are mostly circular in shape. The size of the vessels varied. The maximum length of vessels seen was 88-92µm and the width of the vessels ranged between 77-82.5µm. The diameter of the pith cells observed was 86.5-90µm. The pith rays were comprised of 1-2 rowed circular to polygonal shaped cells. Root hairs were also seen.



a. LM of root showing xylem vessels



b. SEM showing cortex parenchyma and vascular bundles

Fig 15: Skimmia laureola DC

Plant names	Exodermis Length (µm)	Exodermis Width (μm)	Cork Cells Length (μm)	Cork Cells Width (µm)	Cortex Parenchyma Cells Length (µm)	Cortex Parenchyma Cells Width (µm)	Endodermis Length (µm)	Phloem Strand Length (µm)	Xylem Tracheae Diameter (µm)	Vessel Length (µm)	Vessel Width (µm)	Pith Cells Diameter (µm)
Citrus Reticulata	47-50	24-29.5	46-50.5	24.5-28	45-51.5	20-23	40-44.5	65-69	9-13	45-87	52-120	21-43
Citrus Limon	50.5-56.5	27-31	35-37.5	17.5-21	37-44	21.5-29	55-61	89-99	12-17	55-69.5	42-47.5	30-47.5
Citrus Sinensis	70-83.5	45-47.5	47-54	27.5-32	55-61	40-53.5	110-120.5	105-129	26-33	115-127	75-80.5	51-60
Citrus Medica Var Acida	70-90	30-36.5	47-51	30.5- 40.5	75-86.5	54-63	90-110	109-118.5	66-73.5	145-166.5	112-119.5	91-106.5
Citrus Limetta	107-119.5	73-78.5	92-120.5	67-74.5	105.5-116.5	94-103	89-103	91-110	104.5-111.5	152.5-170	110-119.5	101-118
Citrus Grandis	77.5-81.5	45.5-56.5	102-110	80.5-86	90-100	67-73.5	-	87.5-90	100.5-105	99.5-110.5	70-78.5	91.5-108.5
Citrus Aurantium	51.5-55.5	32.5-39	93-99	76-80.5	59.5-64	44.5-60	95-101	60-69	22.5-27.5	75-80	52-67	39-45
Citrus Aurantifolia	107.5-115	47-55.5	86-92.5	63-66	30.5-35	19-21.5	60-64	115-120	15-18	43-47.5	40-45.5	23.5-45
Citrus Paradisi	87-90	53-59	77.5-82	43-47.5	100.5-105	79.5-85	81-94	175-182	29-37	52-60.5	39-44	30.5-37.5
Murraya koenigii Linn.	83-85	37.5-40	62-66.5	28-32	78.5-84	68-74.5	65-69	70-77	87-90.5	31-35.5	41.5-46.5	30.5-34.5
Murraya paniculata Linn.	77-80	49.5-52.5	47.5-53	24-28.5	62.5-68	62.5-68	47.5-51.5	52.5-58	66.5-70	22.5-27	36-40	22-26
Zanthoxylum armatum DC.	99-103.5	40.5-46	49-54.5	30-35.5	50-57	33.5-39	57.5-61	66-70	77-80.5	31-39	42-44.5	30-37.5
Aegle marmelos Linn.	91-95	52.5-56.5	60-68	44.5-48	93.5-97.5	42-49.5	88.5-92.5	86-90	117.5-124	53-59	55-60.5	40.5-47.5
Fortunella japonica	137-145	77.5-87	98-104	69.5-74	143-151.5	72-79	108-115	106-110	144.5-150.5	77-84	79.5-84	62.5-70
Skimmia laureola DC.	122.5-126	69-72	97-102	72-76.5	130.5-137	66-69.5	101.5-107.5	112.5-120	160-165	90.5-94	88-92	77-82.5

Table 1: Quantitative study of various parameters of root transverse section cutting of Rutaceae Species

DISCUSSION

Root anatomy in the Rutaceae family exhibits distinctive features that set it apart from other plant families. The roots typically display adaptations that reflect the diverse habitats in which these plants thrive, ranging from arid regions to moist woodlands. Exploring the intricacies of Rutaceae root anatomy provides valuable insights into their ecological resilience and the mechanisms they employ to adapt to varying environmental conditions.

In the present studies root anatomical characteristics observed corroborate with the findings of Manner *et al.*, 2016. The root characteristics such as exodermis, endodermis, vascular bundles, cortex parenchyma cells, phloem strand, tracheids and vessels were studied. The current studies was focused on these significant root anatomical characteristics which are supportive in identification and delimitation of studied plant taxa.

The external surface of the roots in Rutaceae taxa is enveloped by the exodermis, which can vary in thickness, ranging from thick to thin, and may appear broken or crushed in the transverse sections. Examination of root anatomical features through transverse sectioning of Rutaceae taxa revealed considerable diversity in the exodermis, consisting of anywhere from one to several layers. Citrus reticulata Blanco., Citrus sinensis Linn. Osbeck., Citrus limetta Risso., Murraya koenigi Linn. and Murraya paniculata Linn. had single layer of exodermis whereas 6-9 layers of exodermis were seen in Citrus limon (Linn.) Burm.f., Citrus medica., Citrus paradisi Macfadyen. and Aegle marmelos Linn. The maximum number of exodermis layers almost 12-16 layers was seen in Zanthoxylum armatum DC. The maximum length of exodermis cells was ranged from 137-145µm observed in Fortunella japonica whereas the minimum length of exodermis cells was ranged from 37.5-40µm seen in Murraya koenigi Linn. The maximum width of the exodermis cells was 77.5-87µm seen in Fortunella japonica whereas the minimum width of the exodermis cells was ranged between 24-29.5 seen in Citrus reticulata. These findings were in accordance with the results of Patricia 2012 who also documented multilayers of exodermis, characterized by variations in both the length and width of exodermis cells in Rutaceae taxa. Variations were also observed in the thickness of the exodermis cells. In mostly species the exodermis cells were thick such as Citrus limon, Citrus medica, Citrus limetta, Citrus grandis, Citrus aurantium, Citrus aurantifolia. Citrus paradisi, Murraya koenigi, Murraya paniculata, Fortunella japonica and Skimmia laureola while in other species exodermis cells had thin walls. The occurrence and extent of exodermis or secondary growth in the roots of Rutaceae taxa can differ among various species within the family. Some species may display limited secondary growth or exodermis development in their roots, while others may predominantly maintain a primary growth pattern characteristic of herbaceous plants. (Chase et al., 2019).

Under the outer layer of exodermis, the cork cells and cortex parenchyma cells were present. The maximum length of cork cells observed was 102-110µm in *Citrus grandis* whereas the minimum length of cork cells was ranged between17.5-21µm seen in *Citrus limon*. The specific characteristics of cortex parenchyma cells in Rutaceae may vary among different species within the family. Analyzing these cells can provide insights into the plant's adaptations, nutritional strategies, and overall root anatomy. (Baran and

Ozdemir, 2019). In the present research work distinctions were also observed in the cortex parenchyma cells of Rutaceae taxa. The cortex parenchyma cells were of different shapes and sizes. Mostly polygonal, circular and irregular shaped parenchyma cells were observed. But in some cases different and unique parenchyma cells were also observed, such as elongated shaped parenchyma cells were seen only in *Citrus reticulata* whereas Citrus limon had vasicentric aliform parenchyma cells. According to the studies of Roy, 2016 the vasicentric aliform parenchyma has been reported to be a very advanced condition. The cortex parenchyma cells were composed of many layers. Maximum cortex parenchyma layers were 23-29 observed in Citrus medica and the minimum cortex parenchyma layers were 5-9 in Citrus reticulata. These variations in the cortex parenchyma cells were also observed in the results of Chase et al., 2019 in which they documented the presence of many layered cortex parenchyma cells with distinctions in sizes and shapes in Rutaceae taxa. The cortex parenchyma cells maximum length was ranged between 143-151.5µm observed in Fortunella japonica and the minimum length was ranged between 30.5-35µm seen in Citrus aurantifolia. The maximum width of cortex parenchyma cells ranged between 94-103µm seen in Citrus limetta and the minimum width was ranged between 19-21.5µm in Citrus aurantifolia. These research results were in accordance with the findings of some such other workers. (Ezeabara et al., 2013; Edeoga and Okoli, 2017 and Ilodibia and Okoli, 2016).

In the Rutaceae family, as in other plants, the endodermis acts as a checkpoint for substances entering the vascular system of the root. The selective barrier created by the casparian strip ensures that water and nutrients must pass through the endodermal cells. allowing for regulation and control of nutrient uptake. (Akcin et al., 2018). The endodermis in roots of Rutaceae taxa was also studied. The endodermis observed in the studied taxa was single-multilayered. Single-layered endodermis of root was observed in Citrus reticulata, Zanthoxylum armatum, Aegle marmelos and Skimmia laureola. The maximum length of endodermis cells was observed as 110-120.5 µm in Citrus sinensis whereas the minimum length of the endodermis cells was 40-44.5µm seen in Citrus reticulata. There was also variation in the shapes of the endodermis cells in the studied Rutaceae taxa. Circular-polygonal shaped endodermis cells were observed in Citrus reticulata, Citrus limon, Citrus aurantium, Citrus aurantifolia and Citrus paradisi whereas the irregularpolygonal shaped cells were seen in Citrus sinensis, Citrus limetta and Murraya koenigi. No endodermis cells were observed in the Citrus grandis. Recent studies in developmental and cell biology have started revealing the essential role played by this single layer of endodermis cells as a vital regulatory element in root growth, tissue organization, and nutrient transport. (Chase et al., 2019).

Vascular bundles are complex structures found in the vascular tissues of higher plants. These bundles play a crucial role in the transport of water, nutrients, and sugars throughout the plant. The organization and size of vascular bundles can vary among different plant species and organs. (Swingle and Reece, 2017). In the present research work the maximum vascular cylinder diameter observed was 200-209µm observed in *Citrus paradisi* and the minimum vascular cylinder diameter was 52.5-58µm seen in *Murraya paniculata*. The two main types of vascular tissues are xylem and phloem, and

vascular bundles contain various arrangements of these tissues. The well-established and well developed phloem strands were seen in the present studies in the studied taxa of Rutaceae. The maximum length of the phloem strand was also observed in Citrus paradisi which is measured as 175-182µm. Whereas the minimum length of the phloem strand was 60-69µm seen in Citrus aurantium. Tracheids and vessels are the conducting elements of xylem responsible for the transport of water and minerals. Vessels are found in angiosperms, while tracheids are present in both angiosperms and gymnosperms. In the present studies variation was found in size of tracheids among different species. The maximum diameter of tracheids found was 104.5-111.5µm seen in Citrus limetta and the minimum diameter of tracheids was 9-13 µm observed in *Citrus reticulata*. While the maximum length of vessels was 152.5-170µm found in Citrus limetta whereas the minimum length of vessels was found as 42-44.5µm in Zanthoxylum armatum. The variation was also seen in vessels width in the studied taxa. The maximum width of vessels was 112-119.5µm observed in Citrus medica whereas the minimum width of vessels was 30-37.5µm in Zanthoxylum armatum. The variations in the sizes of tracheids and vessels were also seen in the work of Atasagun et al., 2015.

This investigation was aimed to delve into the structural components of Rutaceae roots, shedding light on the specialized tissues, root architecture, and adaptive features that contribute to the overall success and versatility of these fascinating plants within the botanical world. By examining the root anatomy of the Rutaceae family, we gain a deeper appreciation for the remarkable strategies these plants have evolved to ensure their survival and thriving across diverse ecosystems.

CONCLUSION

This study is the first to examine root transverse sections using both light and scanning electron microscopy. The anatomical analysis of root transverse sections in Rutaceae taxa has revealed distinct morphological features essential for their identification and classification. The anatomical study of root transverse sections in Rutaceae species not only enriches scientific understanding but also provides practical benefits for plant cultivation and management, ultimately supporting the sustainable use of these species in various agricultural and horticultural contexts. This is particularly useful in distinguishing closely related species that may appear similar in other aspects.

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