IJABR Vol. 17 (1): 337 - 356 (2026)

Original Article

Scanning Electron Microscopic Investigation of Leaf, Stem, and Root Micromorphology of *Amaranthus blitum* L. (Amaranthaceae) (Purple amaranth): Taxonomic and Ecological Implications

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Submitted: Febuary 2026; Accepted: April 2026; Published: June 2026

ABSTRACT

Amaranthus blitum L. (Purple amaranth) is an important leafy vegetable and medicinal herb widely distributed in tropical and subtropical regions. Despite its nutritional, ethnobotanical, and ecological importance, detailed micromorphological investigations of its vegetative organs remain limited. This study employed scanning electron microscopy (SEM) to investigate the leaf, stem, and root anatomy of *A. blitum* with emphasis on epidermal characteristics, vascular organization, tissue differentiation, and adaptive structural features of taxonomic significance. Fresh plant materials were processed using standard SEM preparation procedures and examined at varying magnifications. The leaf epidermis revealed predominantly anomocytic stomata distributed on both adaxial and abaxial surfaces, with greater stomatal abundance on the abaxial epidermis. Epidermal cells were polygonal to irregular with undulate to sinuous anticlinal walls. Simple non-glandular trichomes and fine cuticular ornamentations including striations and granular deposits were observed on both surfaces. Stem anatomy showed a well-organized herbaceous dicotyledonous structure characterized by compact epidermal tissues, abundant parenchymatous cortex, discrete vascular bundles, large, lignified xylem vessels, and supportive fibrous tissues. The root anatomy revealed extensive parenchymatous tissues with conspicuous intercellular spaces, large conducting xylem vessels, and scalariform secondary wall thickenings indicative of efficient conduction and structural reinforcement. The observed micromorphological features demonstrate important adaptive modifications associated with water conservation, gaseous exchange, mechanical support, storage, and environmental tolerance. Diagnostic characters such as anomocytic stomata, non-glandular trichomes, scalariform xylem thickenings, and cuticular ornamentation further support the taxonomic relevance of SEM in anatomical characterization of *Amaranthus* species. The study provides valuable baseline data for systematic studies, ecological interpretation, and comparative anatomical investigations within the family Amaranthaceae.

Keywords: *Amaranthus blitum*; scanning electron microscopy; micromorphology; stomata; trichomes; vascular tissues; epidermal anatomy

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INTRODUCTION

The genus *Amaranthus* L. (Amaranthaceae) comprises approximately 60–70 species that are widely distributed throughout tropical, subtropical, and temperate regions of the world. Members of the genus exhibit remarkable ecological adaptability and are cultivated or occur naturally as leafy vegetables, pseudocereals, ornamental plants, forage crops, and medicinal herbs. Many species are recognized for their high nutritional value owing to their rich contents of proteins, essential amino acids, dietary fibre, vitamins, minerals, and bioactive phytochemicals, making them important components of food security and traditional healthcare systems in many developing countries **[1,2].** Among the edible species, *Amaranthus blitum* L. (purple amaranth) is an annual herb that is widely consumed as a leafy vegetable and has long been employed in ethnomedicine for the management of several ailments. The species is valued for its rapid growth, adaptability to diverse environmental conditions, and ability to thrive under marginal agricultural systems. Previous studies have demonstrated that *A. blitum* contains appreciable quantities of phenolic compounds, flavonoids, carotenoids, vitamins, minerals, and antioxidant constituents that contribute to its nutritional and therapeutic importance **[2,3].**

Despite its economic significance, species identification within *Amaranthus* remains challenging because of extensive morphological variation, phenotypic plasticity, natural hybridization, polyploidy, and the strong influence of environmental conditions on vegetative characters. Reliance solely on macromorphological traits frequently results in taxonomic ambiguity, particularly among closely related species. Consequently, the integration of anatomical and micromorphological characters has become increasingly important in resolving taxonomic relationships and improving species delimitation within the genus **[4,5].** Plant anatomical characteristics are generally considered more stable than external morphological traits because they are less influenced by environmental fluctuations. Micromorphological features of the leaf epidermis, including stomatal architecture, epidermal cell configuration, anticlinal wall patterns, trichome morphology, and cuticular ornamentation, have proven particularly valuable in systematic investigations of angiosperms. Likewise, anatomical characteristics of stems and roots, such as vascular bundle arrangement, xylem vessel morphology, cortical organization, and sclerenchymatous tissues, provide additional diagnostic evidence for taxonomy while simultaneously reflecting ecological adaptations and physiological functions **[5–8].**

Scanning electron microscopy (SEM) has become one of the most effective techniques for investigating plant surface micromorphology because it provides high-resolution, three-dimensional images of epidermal and internal anatomical structures. Compared with conventional light microscopy, SEM enables detailed visualization of stomatal complexes, trichomes, cuticular sculpturing, epidermal cell boundaries, vascular tissues, and secondary wall thickenings with exceptional clarity. Consequently, SEM has been widely adopted in systematic botany, comparative anatomy, developmental biology, and ecological studies to identify taxonomically informative characters and to improve understanding of plant structural adaptations **[9–11]**.

Micromorphological features also possess considerable ecological significance. Stomatal density, distribution, and architecture influence transpiration, photosynthetic efficiency, and gaseous exchange, whereas trichomes contribute to protection against herbivory, excessive solar radiation, pathogen invasion, and water loss. Similarly, xylem vessel diameter, secondary wall thickening, and vascular organization determine the efficiency of water transport, hydraulic conductivity, and mechanical support. Cuticular ornamentation further enhances resistance to desiccation and environmental stress, thereby improving plant survival under fluctuating environmental conditions **[8,12–15]**. Although numerous investigations have focused on the nutritional composition, phytochemistry, pharmacological activities, and agronomic performance of *Amaranthus* species, comparatively little information is available regarding the ultrastructural anatomy of *A. blitum*, particularly in tropical African populations. Comprehensive SEM-based studies describing the micromorphology of its vegetative organs remain scarce. Such information is important not only for species identification but also for understanding adaptive structural modifications associated with environmental tolerance and ecological performance.

Therefore, the present study employed scanning electron microscopy to investigate the leaf, stem, and root micromorphology of *Amaranthus blitum*. Particular emphasis was placed on epidermal characteristics, stomatal architecture, trichome morphology, cuticular ornamentation, tissue differentiation, vascular organization, and adaptive structural features. The findings provide valuable diagnostic characters for taxonomic identification, contribute to comparative anatomical studies within the Amaranthaceae, and enhance current understanding of the ecological and functional significance of vegetative micromorphology in *Amaranthus*.

MATERIALS AND METHODS

Study Area and Plant Material

Healthy plants of *Amaranthus blitum* L. were collected from naturally occurring populations within Ilorin, Kwara State, Nigeria, during the active vegetative growth stage. Young, fully expanded leaves, succulent stems, and healthy roots were selected to ensure optimum preservation of anatomical features. Plant identification and authentication were carried out in the Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Nigeria, using standard taxonomic keys and authenticated floristic literature. Voucher specimens were prepared, assigned accession numbers, and deposited in the departmental herbarium for future reference.


Fig. 1: Photograph of *Amaranthus blitum*

Sample Preparation for Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was conducted at the Microscopy and Microanalysis Unit (MMU), University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa. Fresh leaf, stem, and root tissues were excised using sterile stainless-steel blades. Small tissue segments (approximately 5–10 mm^2) were prepared from both the adaxial and abaxial leaf surfaces, while transverse and longitudinal sections of stems and roots were obtained using sharp razor blades to expose internal anatomical structures **[19,20]**.

The specimens were gently rinsed with distilled water to remove adhering soil particles, dust, and other contaminants without damaging the epidermal surface. Samples were immediately immersed in 2.5% glutaraldehyde prepared in 0.1 M phosphate buffer (pH 7.2) and fixed for 12–24 h at room temperature to preserve cellular ultrastructure and minimize tissue degradation **[21]**. Following primary fixation, the specimens were washed three times in fresh phosphate buffer (10 min each) to remove residual fixative before dehydration.

Dehydration and Drying

Fixed tissues were dehydrated through a graded ethanol series consisting of 30%, 50%, 70%, 90%, 95%, and 100% ethanol. Samples remained in each concentration for approximately 15–20 min to ensure gradual removal of cellular water and minimize tissue distortion **[21,22]**. Absolute ethanol was replaced twice to achieve complete dehydration. The dehydrated specimens were subsequently dried by critical point drying whenever available or by controlled air-drying when critical point drying facilities were unavailable. Critical point drying was preferred because it minimizes tissue shrinkage and preserves delicate surface structures, particularly stomata, trichomes, and cuticular ornamentation **[20,23]**.

Mounting and Sputter Coating

Completely dried specimens were mounted on aluminium SEM stubs using double-sided conductive carbon adhesive tabs. Leaf samples were mounted separately to expose both adaxial and abaxial epidermal surfaces, whereas stem and root samples were oriented to reveal transverse and longitudinal sections. Mounted specimens were sputter-coated with a thin conductive layer of gold using an automated sputter coater. Gold coating improved electrical conductivity, minimized charging artefacts during electron beam exposure, and enhanced image quality and resolution **[20]**.

Scanning Electron Microscopy

The coated specimens were examined using a scanning electron microscope operated under appropriate accelerating voltage and high-vacuum conditions according to the manufacturer's operating guidelines. Digital micrographs were captured at multiple magnifications depending on the anatomical structures under investigation. Leaf observations focused on epidermal cell morphology, stomatal type and distribution, guard cell architecture, anticlinal wall configuration, trichome morphology, and cuticular ornamentation. Stem observations included epidermal organization, cortical tissues, vascular bundle arrangement, xylem vessel morphology, phloem tissues, supporting fibres, and ground tissues. Root observations concentrated on cortical organization, vascular cylinder, xylem vessel architecture, secondary wall thickening, intercellular spaces, and surface micromorphology.

Anatomical Characterization

Micromorphological observations were interpreted using internationally accepted anatomical terminology following the classical works of Metcalfe and Chalk **[5]**, Esau **[7]**, Fahn **[8]**, Barthlott **[16]**, and Barthlott et al. **[18].** Diagnostic characters recorded included stomatal type, stomatal distribution, epidermal cell shape, anticlinal wall pattern, trichome type, cuticular ornamentation, vascular bundle arrangement, xylem vessel characteristics, cortical organization, and intercellular spaces. Descriptions were compared with published anatomical reports on members of the family Amaranthaceae to determine their systematic significance.

Image Processing and Documentation

Representative SEM micrographs showing diagnostic anatomical features were selected for detailed analysis. Images were examined for structural integrity, clarity, and reproducibility before interpretation. Observed anatomical structures were described according to standard botanical terminology, and their probable physiological, ecological, and taxonomic significance was evaluated using published literature **[5–18]**.

Data Analysis

This investigation was primarily descriptive and comparative. Qualitative anatomical characters were recorded for each vegetative organ and compared with published reports on *Amaranthus* and related genera within the Amaranthaceae. Observed characters were interpreted with respect to taxonomic significance, ecological adaptation, physiological function, and structural specialization. Where appropriate, similarities and differences among leaf, stem, and root tissues were summarized in comparative tables.

RESULTS

Scanning Electron Microscopic Analysis of the Leaf Epidermis of *Amaranthus blitum*

Scanning electron microscopy (SEM) revealed distinct micromorphological features of the adaxial and abaxial leaf epidermis of *Amaranthus blitum*. The observed characters included stomatal architecture, epidermal cell morphology, anticlinal wall configuration, trichome morphology, and cuticular ornamentation, all of which are of considerable taxonomic and ecological significance.

Abaxial Leaf Surface

The abaxial epidermis possessed numerous stomata that were predominantly anomocytic, with guard cells surrounded by ordinary epidermal cells lacking specialized subsidiary cells (Fig. 1). The stomatal pores were elliptical to narrowly oval, while some guard cells appeared slightly sunken below the epidermal surface. A higher stomatal density was observed on the abaxial surface than on the adaxial surface, indicating a predominantly hypostomatic leaf condition.

The epidermal cells were irregularly polygonal to elongated and exhibited slightly undulate anticlinal walls (Fig. 2). Fine cuticular ridges surrounding the stomatal complexes and occasional epicuticular deposits were observed. These cuticular modifications contributed to the smooth to slightly wrinkled appearance of the epidermis. Simple unicellular non-glandular trichomes occurred abundantly on the abaxial surface (Fig. 3). The trichomes were elongated, unbranched, and tapering, with smooth to slightly roughened surfaces. Some collapsed trichomes were observed, probably resulting from dehydration during specimen preparation.

Adaxial Leaf Surface

The adaxial epidermis exhibited micromorphological characteristics similar to those of the abaxial surface but with notable differences in stomatal frequency and surface ornamentation. Anomocytic stomata were present but occurred less frequently than on the lower epidermis (Fig. 4). The guard cells were oval in shape, and several stomata were partially sunken within the epidermis and surrounded by radiating cuticular folds.

The epidermal cells were polygonal to irregular and more compactly arranged than those of the abaxial epidermis (Fig. 5). Their anticlinal walls were distinctly sinuous, producing an interlocking cell pattern that enhanced tissue cohesion. Non-glandular trichomes similar to those observed on the abaxial surface were also present (Fig. 6). These structures appeared elongated, tapering, and occasionally coated with granular epicuticular deposits. The adaxial cuticle exhibited greater sculpturing than the lower epidermis and was characterized by fine striations, rugose surfaces, and parallel cuticular folds surrounding the stomatal complexes.

Comparative Leaf Epidermal Characteristics

Although both epidermal surfaces possessed anomocytic stomata, polygonal epidermal cells, and simple non-glandular trichomes, clear differences were observed between the two surfaces (Table 1). The abaxial surface exhibited higher stomatal density, relatively smoother cuticle, flatter epidermal relief, and less pronounced anticlinal wall undulation. Conversely, the adaxial surface showed lower stomatal density, more conspicuous trichomes, strongly sinuous anticlinal walls, and greater cuticular sculpturing. These observations demonstrate structural differentiation between the two epidermal surfaces and indicate functional specialization associated with transpiration control and environmental adaptation.

Table 1: Comparative scanning electron microscopic (SEM) characteristics of the adaxial and abaxial leaf epidermis of *Amaranthus blitum* L., highlighting diagnostic micromorphological features and their taxonomic and ecological significance.

Character	Abaxial Surface	Adaxial Surface	Taxonomic/Ecological Significance
Stomatal distribution	More abundant	Less abundant	Higher stomatal density on the abaxial surface reduces transpiration while maintaining efficient gaseous exchange.
Stomatal type	Anomocytic	Anomocytic	Diagnostic character supporting placement within the Amaranthaceae.
Guard cell morphology	Elliptical to narrowly oval; some slightly sunken	Oval; some partially sunken	Sunken stomata enhance water conservation under environmental stress.
Epidermal cell shape	Irregularly polygonal to elongated	Polygonal to irregular, more compact	Useful diagnostic character for species identification.
Epidermal cell arrangement	Loosely arranged	Compactly arranged	Reflects functional differentiation between the two epidermal surfaces.
Anticlinal wall pattern	Slightly undulate	Strongly sinuous to undulate	Sinuous walls increase tissue cohesion and mechanical stability.
Trichome type	Simple, unicellular, non-glandular	Simple, non-glandular (predominantly unicellular)	Provides mechanical protection and has taxonomic value.
Trichome abundance	Moderate	More conspicuous	Enhances protection against herbivory, excessive irradiance, and water loss.
Cuticular ornamentation	Smooth to slightly wrinkled with fine striations	Rugose with distinct striations and cuticular folds	Contributes to environmental protection and species differentiation.
Epicuticular deposits	Occasional granular deposits	More conspicuous granular deposits	May enhance resistance to desiccation and pathogen invasion.
Surface relief	Relatively flat	More sculptured	Indicates functional specialization of the upper epidermis.
Overall adaptive significance	Optimized for transpiration regulation and gaseous exchange	Optimized for mechanical protection and light interception	Demonstrates complementary functional specialization of the two epidermal surfaces.

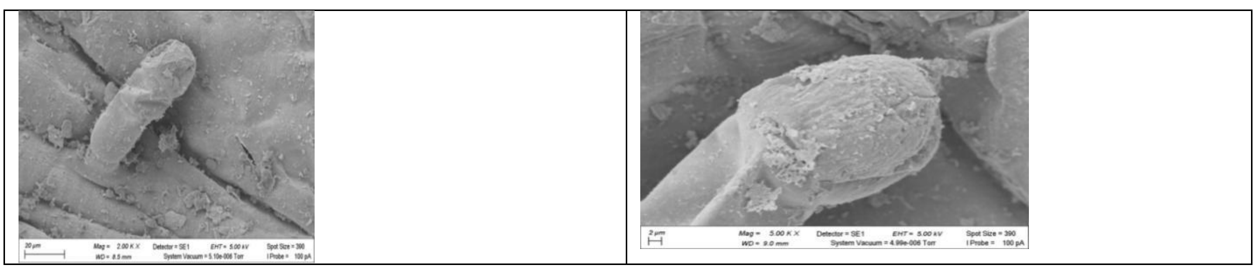

Fig. 2: Micrographs of abaxial leaf surface simple unicellular non-glandular trichomes

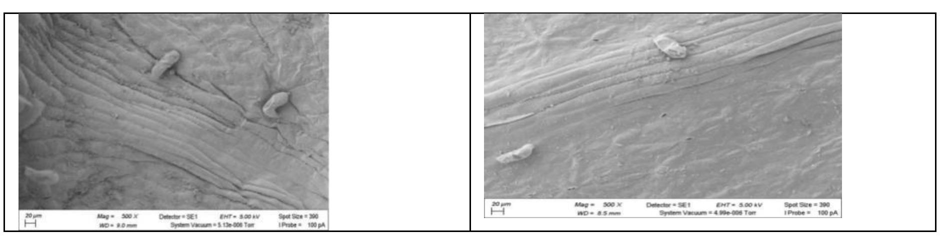

Fig. 3: Leaf epidermal cells of abaxial surface of *Amaranthus blitum*

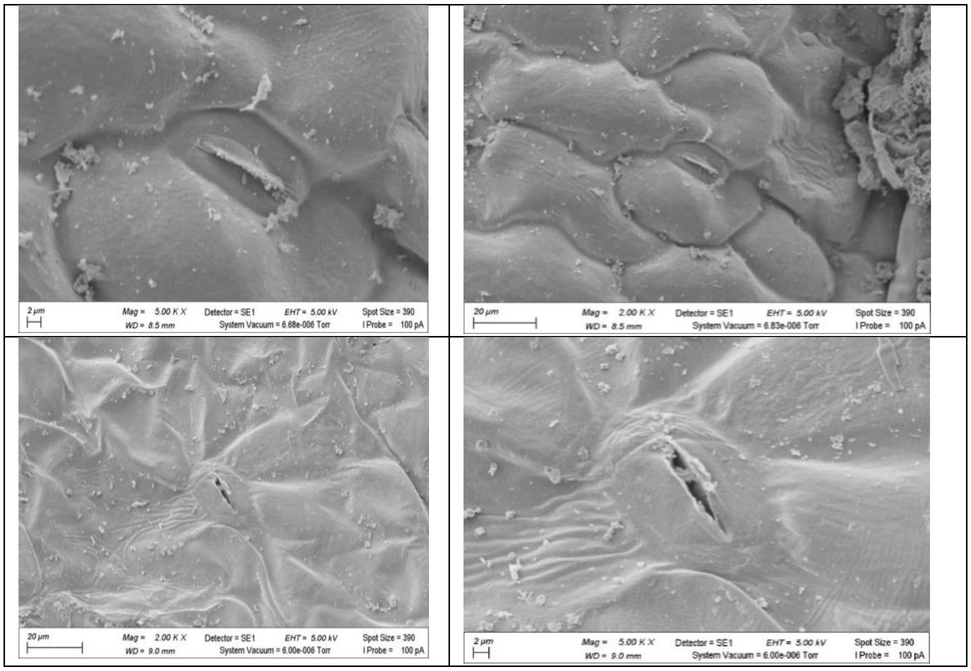

Fig. 4: Micrographs of adaxial leaf surface of *Amaranthus blitum* showing anomocytic stomata

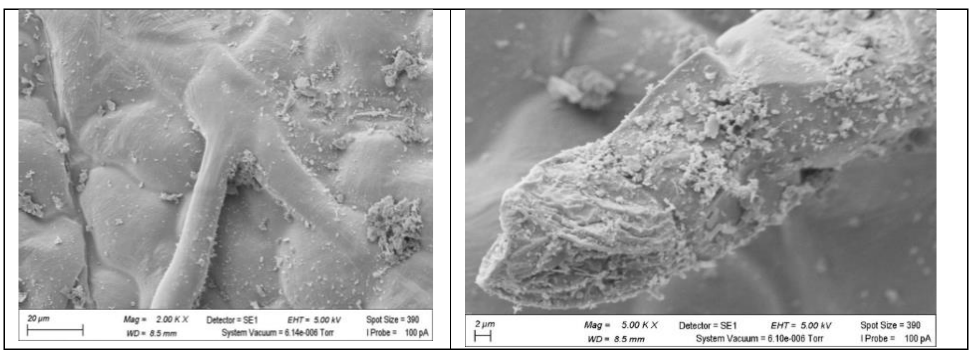

Fig. 5: Micrographs of adaxial epidermis showing non-glandular trichomes

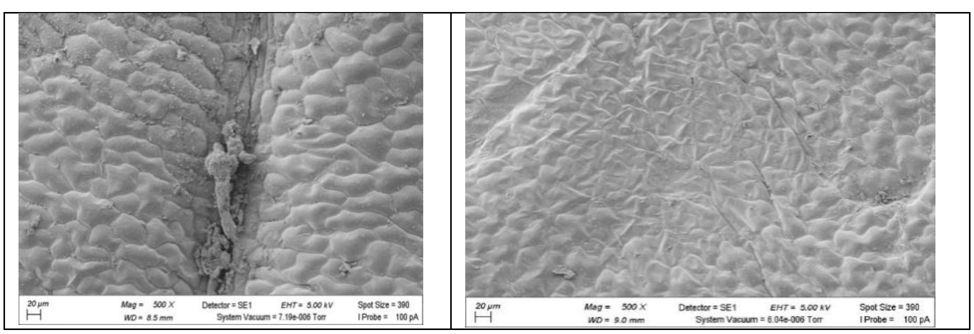

Fig. 6: Micrographs of epidermal cells on the adaxial surface of *Amaranthus blitum*

Stem Anatomy

SEM examination of transverse and longitudinal stem sections demonstrated a typical herbaceous dicotyledonous organization consisting of a compact epidermis, cortical parenchyma, discrete vascular bundles, and abundant ground tissue (Fig. 7). The epidermis formed a continuous protective layer covered by a thin cuticle. Beneath the epidermis, the cortex consisted predominantly of thin-walled parenchyma with conspicuous intercellular spaces.

Vascular Bundle Organisation

Discrete collateral vascular bundles were embedded within the ground tissue (Fig. 8). Each bundle consisted of well-developed xylem and phloem tissues associated with supportive fibres. The xylem vessels possessed thick lignified secondary walls and wide lumina, indicating specialization for efficient water transport and mechanical support.

Xylem Vessel Morphology

SEM micrographs revealed large polygonal xylem vessels with conspicuous secondary wall thickening (Fig. 9). The vessels exhibited wide conducting lumina, thick lignified walls, compact arrangement, and interconnected conducting channels. Occasional ruptured vessels were observed and were interpreted as preparation artefacts.

Ground Tissue

The ground tissue consisted mainly of large thin-walled parenchyma cells with conspicuous vacuoles and intercellular spaces. These tissues occupied much of the stem interior and are consistent with functions in storage, gaseous exchange, metabolite transport, and maintenance of stem flexibility.

Stem Surface Characteristics

The external stem surface exhibited fine longitudinal striations and a relatively smooth cuticle (Fig. 10). Elliptical stomata occurred sporadically on the epidermis, while mild cuticular ornamentation consisting of granular deposits and shallow wrinkles was also observed.

Overall, the stem anatomy of *Amaranthus blitum* was characterized by compact epidermis, abundant cortical parenchyma, discrete collateral vascular bundles, large lignified xylem vessels, supportive sclerenchymatous fibres, and smooth cuticular surface with scattered stomata.

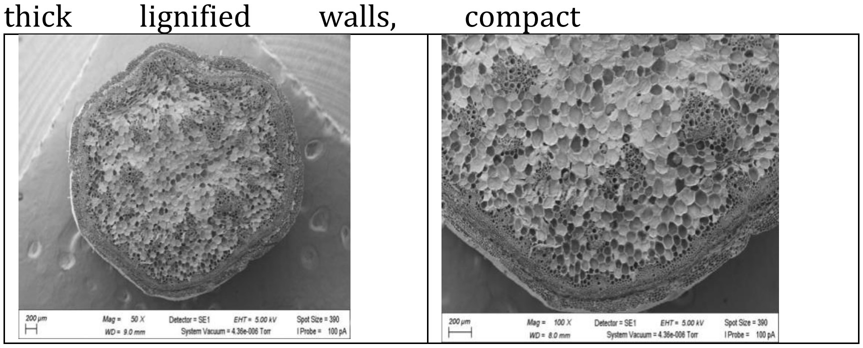

Fig. 7: Micrographs of the stem cross sections revealed the general internal organization of the stem

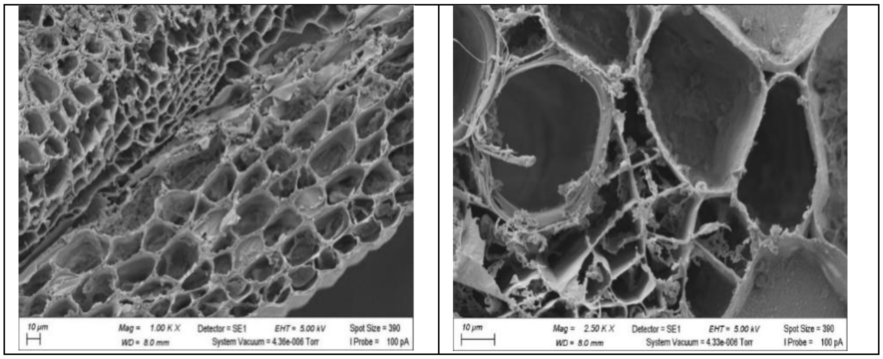

Fig. 8: Vascular tissues in the stem sections showing elongated and thickened conducting elements representing xylem and phloem tissues organized into vascular bundles

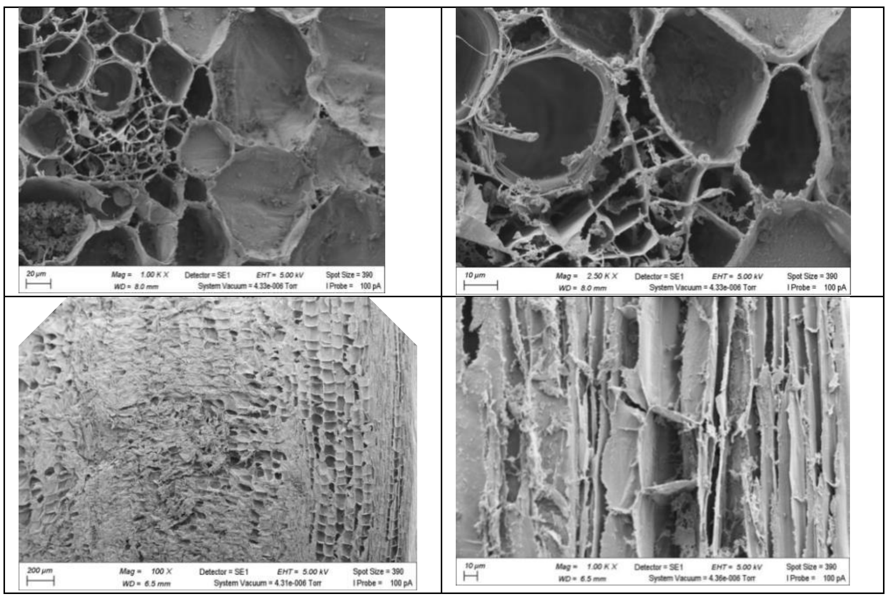

Fig. 9: Micrographs of large xylem vessels with wide lumina and polygonal outlines

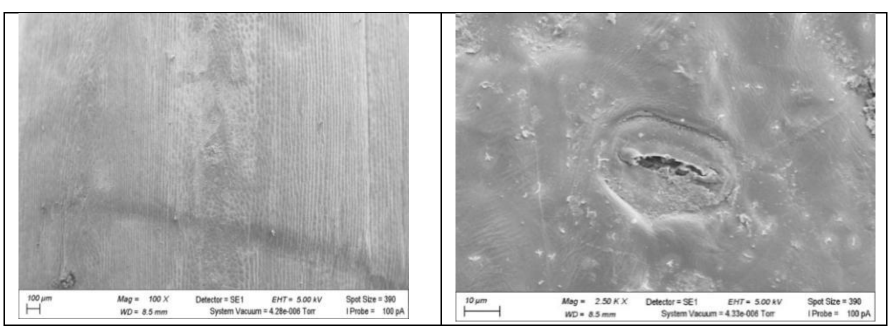

Fig. 10: Transverse section micrographs revealing the external stem surface with epidermal features and stomatal openings

Root Anatomy

SEM observations revealed a well-organized root structure consisting of an outer protective layer, extensive cortical tissues, a compact vascular cylinder, and abundant conducting elements (Fig. 11).

Cortical Organisation

The cortex consisted predominantly of thin-walled parenchyma with numerous intercellular spaces (Fig. 12). The cells were irregularly polygonal to rounded and formed a loosely arranged tissue network indicative of active storage and internal aeration.

Vascular Cylinder

Large conducting elements occurred within the central vascular cylinder (Fig. 13). The xylem vessels possessed thick lignified secondary walls, wide lumina, polygonal outlines, and compact arrangement. Smaller surrounding cells represented phloem tissues and associated parenchyma.

Longitudinal Conducting Elements

Longitudinal sections revealed elongated xylem vessels exhibiting distinct
.
scalariform secondary wall thickening (Fig. 14). The ladder-like wall thickenings provide structural reinforcement while maintaining efficient water conduction.

Ground Tissues

Ground tissues surrounding the vascular cylinder consisted mainly of elongated parenchyma with conspicuous intercellular spaces (Fig. 15). These tissues appeared metabolically active and are consistent with storage and internal transport functions.

Root Surface

The outer surface of the root exhibited a rough texture with fine granular ornamentation (Fig. 16). Unlike the stem and leaf epidermis, stomata were absent, reflecting the absorptive rather than photosynthetic function of the root.

Storage Cells

Large rounded storage cells were observed within the cortical and vascular regions (Fig. 17). These cells possessed large lumina, relatively thick walls, compact arrangement, and close association with vascular tissues

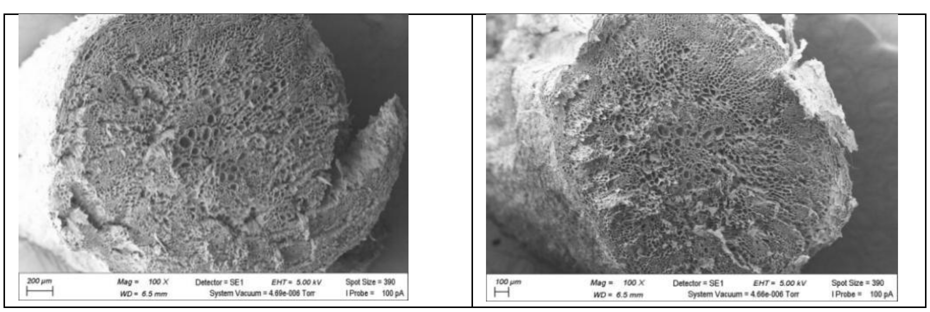

Fig. 13: Micrographs of internal structure of root of *Amaranthus blitum*

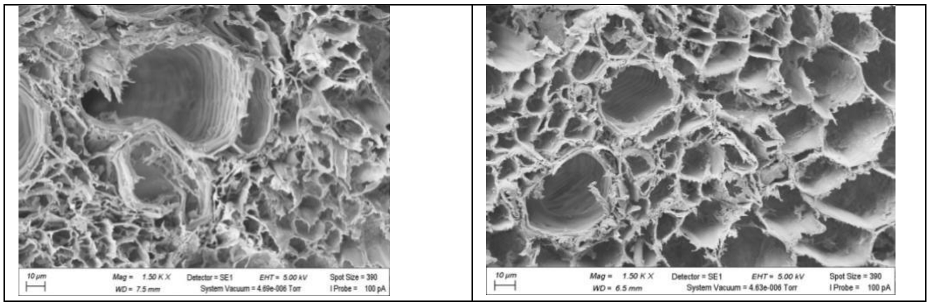

Fig. 14: Micrographs of cortical region showing predominantly of thin-walled parenchymatous cells with conspicuous intercellular spaces

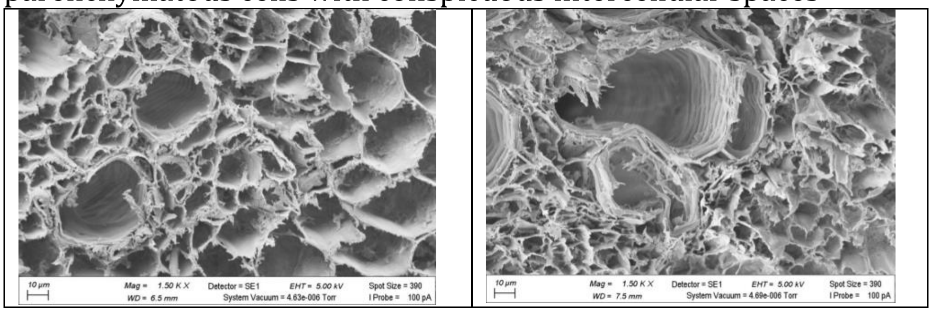

Fig. 15: Micrgraphs of vascular tissues showing large conducting elements embedded within surrounding parenchymatous tissues

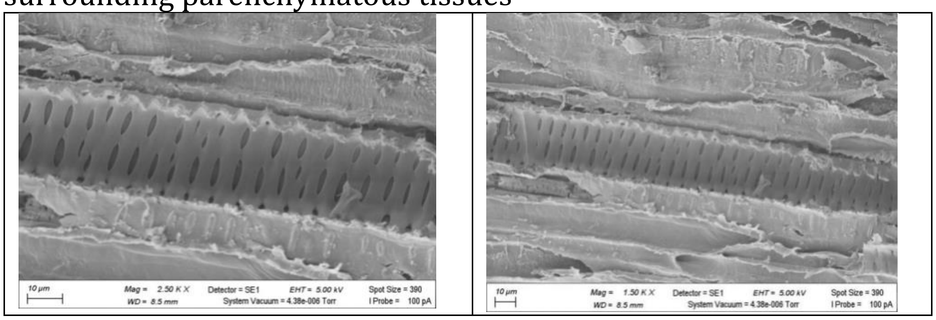

Fig. 16: Transverse section of root showing elongated conducting tissues arranged longitudinally within the root axis

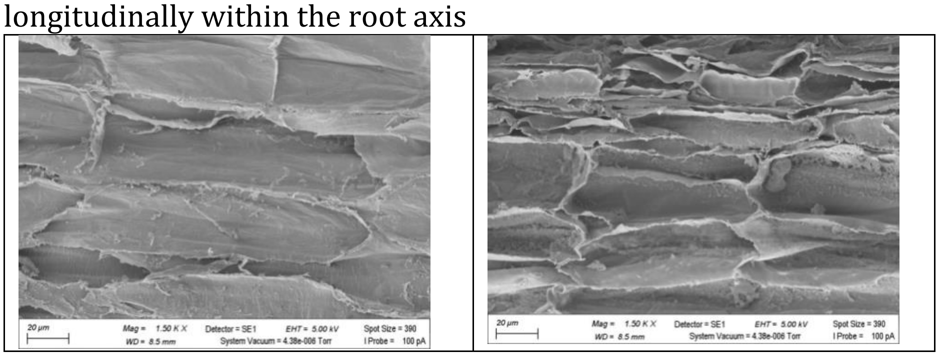

Fig. 17: Micrographs transverse section of root showing the surrounding ground tissues composed mainly of elongated and polygonal parenchymatous cells

Comparative Summary of Vegetative Organs

Comparative SEM analysis demonstrated marked anatomical specialization among the three vegetative organs (Table 2). The leaf was characterized primarily by epidermal diagnostic characters, including anomocytic stomata, non-glandular trichomes, and cuticular ornamentation.

The stem exhibited well-developed collateral vascular bundles, abundant parenchyma, and large lignified xylem vessels that provide both mechanical support and efficient hydraulic conductivity. The root possessed extensive cortical tissues, conspicuous intercellular spaces, compact vascular organization, and scalariform xylem vessel thickenings associated with water uptake and transport.

Overall, the observed micromorphological features provide valuable diagnostic characters for the identification of *Amaranthus blitum* and demonstrate structural adaptations that enhance water conservation, mechanical support, storage, gaseous exchange, and environmental tolerance

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Table 2: Comparative diagnostic micromorphological characteristics of the leaf, stem and root of *Amaranthus blitum* L. revealed by scanning electron microscopy (SEM).

Character	Leaf	Stem	Root	Taxonomic and Functional Significance
Protective tissue	Single-layered epidermis with cuticle	Compact epidermis with thin cuticle	Epidermis/periderm forming protective outer layer	Protects against mechanical injury, pathogens, and excessive water loss.
Surface ornamentation	Smooth to rugose with fine striations and cuticular folds	Fine longitudinal striations with slight wrinkling	Rough surface with fine granular ornamentation	Useful micromorphological character for species identification and ecological adaptation.
Stomata	Numerous, predominantly anomocytic	Scattered elliptical stomata	Absent	Distribution reflects organ-specific physiological functions.
Stomatal abundance	Higher on abaxial surface; lower on adaxial surface	Low frequency	Absent	Indicates adaptation for efficient gaseous exchange and transpiration regulation.
Stomatal type	Anomocytic	Anomocytic	Not applicable	Diagnostic feature supporting placement within Amaranthaceae.
Epidermal cell shape	Polygonal to irregular	Elongated and compact	Irregular outer cells	Stable taxonomic character useful in comparative anatomy.
Anticlinal wall pattern	Slightly undulate to strongly sinuous	Indistinct	Not evident	Enhances epidermal cohesion and mechanical strength.
Trichomes	Simple, unicellular, non-glandular	Absent	Absent	Important diagnostic feature with protective ecological functions.
Cuticle	Well developed with distinct ornamentation	Thin, continuous	Thin or absent externally	Contributes to water conservation and environmental protection.
Cortex	Not differentiated	Extensive parenchymatous cortex with intercellular spaces	Broad cortex composed of thin-walled parenchyma	Provides storage, aeration, and metabolic support.
Ground tissue	Mesophyll tissues	Large parenchymatous ground tissue	Extensive parenchymatous tissues	Responsible for storage of water, carbohydrates, and metabolites.
Vascular organization	Leaf veins with conducting tissues	Discrete collateral vascular bundles	Central vascular cylinder	Characteristic arrangement supporting taxonomic placement.
Xylem vessels	Present within vascular bundles	Large lignified vessels with wide lumina	Large lignified vessels with scalariform thickening	Efficient water transport and mechanical reinforcement.
Secondary wall thickening	Not conspicuous	Strong lignification	Scalariform secondary wall thickening	Enhances hydraulic efficiency and prevents vessel collapse.
Mechanical tissues	Limited	Sclerenchymatous fibres associated with vascular bundles	Mainly lignified vascular tissues	Provides structural support for the plant body.

Character	Leaf	Stem	Root	Taxonomic and Functional Significance
Intercellular spaces	Minimal	Moderate within cortex	Conspicuous within cortex	Facilitates gaseous exchange and internal aeration.
Storage tissues	Mesophyll parenchyma	Cortical and ground parenchyma	Cortical and vascular parenchyma	Supports nutrient and water storage.
Principal physiological function	Photosynthesis, transpiration, gaseous exchange	Mechanical support and transport	Water absorption and mineral conduction	Demonstrates structural specialization among vegetative organs.
Major adaptive features	Hypostomatic tendency, trichomes, cuticular ornamentation	Lignified vascular bundles and supportive fibres	Scalariform xylem vessels and extensive cortex	Improves drought tolerance, hydraulic conductivity, and environmental adaptation.
Taxonomic importance	High	Moderate to High	High	Combined vegetative characters provide reliable diagnostic features for species identification within *Amaranthus*.

Comparative examination of the vegetative organs demonstrated clear structural specialization associated with their respective physiological functions. The leaf was characterized primarily by epidermal features such as anomocytic stomata, non-glandular trichomes, and distinct cuticular ornamentation that optimize photosynthesis, gaseous exchange, and transpiration control. In contrast, the stem exhibited extensive parenchymatous tissues, discrete collateral vascular bundles, large, lignified xylem vessels, and supportive sclerenchymatous fibres, reflecting its dual role in mechanical support and long-distance transport. The root possessed a broad parenchymatous cortex, conspicuous intercellular spaces, compact vascular tissues, and scalariform xylem vessel thickenings, indicating specialization for water absorption, mineral conduction, storage, and internal aeration. Collectively, these organ-specific micromorphological features provide a comprehensive set of diagnostic characters that enhance the taxonomic characterization of *Amaranthus blitum* and demonstrate structural adaptations to terrestrial environments.

DISCUSSION

The present investigation provides a comprehensive scanning electron microscopic (SEM) characterization of the vegetative organs of *Amaranthus blitum* L. The study revealed several diagnostic micromorphological features of the leaf, stem, and root that are taxonomically informative and ecologically significant. These include the occurrence of anomocytic stomata, polygonal epidermal cells with undulate to sinuous anticlinal walls, simple non-glandular trichomes, distinct cuticular ornamentation, well-developed collateral vascular bundles, large lignified xylem vessels, and scalariform secondary wall thickenings. Collectively, these anatomical attributes support the systematic placement of *A. blitum* within the Amaranthaceae and demonstrate structural adaptations that facilitate survival under diverse environmental conditions.

Leaf Epidermal Micromorphology

The predominance of anomocytic stomata on both leaf surfaces is consistent with previous anatomical studies of

Amaranthus and other genera within the Amaranthaceae, where this stomatal type is regarded as one of the most stable taxonomic characters for species identification **[10,11]**. Although stomatal morphology alone may not completely resolve species boundaries, its integration with other epidermal characters considerably enhances taxonomic discrimination within morphologically similar taxa.

The greater stomatal density observed on the abaxial surface indicates a predominantly hypostomatic leaf condition, a feature commonly associated with improved regulation of transpiration. Concentration of stomata on the lower epidermis reduces direct exposure to solar radiation and consequently minimizes evaporative water loss while maintaining efficient gaseous exchange. The occurrence of slightly sunken stomata further suggests an adaptive response to periodic water stress, as recessed stomatal complexes create localized humid microenvironments that reduce transpiration. Similar xeromorphic adaptations have been reported in numerous herbaceous dicotyledons occupying seasonally dry habitats **[8,14,15]**.

The polygonal epidermal cells and undulate anticlinal walls observed in the present study are characteristic of many dicotyledonous taxa. Interlocking epidermal cells increase tissue cohesion and provide mechanical stability during leaf expansion while allowing sufficient flexibility to withstand environmental stress. Such epidermal configurations have consistently been recognized as reliable micromorphological markers in comparative plant anatomy and systematic botany **[5,6]**.

Simple non-glandular trichomes occurred on both epidermal surfaces and constituted another important diagnostic character. In addition to their systematic value, these structures perform multiple ecological functions, including protection against herbivores, reduction of boundary-layer conductance, moderation of leaf temperature, interception of ultraviolet radiation, and reduction of water loss. Their occurrence on both leaf surfaces suggests that *A. blitum* possesses a multifunctional protective system that enhances survival under fluctuating environmental conditions **[12]**.

Cuticular ornamentation varied from relatively smooth surfaces on the abaxial epidermis to more conspicuous striations and rugosity on the adaxial surface. Such differences likely reflect functional specialization between the two epidermal surfaces. Cuticular sculpturing has been shown to influence wettability, light reflectance, self-cleaning capacity, and resistance to pathogen invasion. These characteristics contribute significantly to the adaptive success of terrestrial plants exposed to varying levels of irradiance and atmospheric moisture **[18]**.

Stem Anatomy and Functional Adaptation

SEM examination demonstrated that the stem possesses the typical anatomical organization of a herbaceous dicotyledon, consisting of a continuous epidermis, extensive cortical parenchyma, discrete collateral vascular bundles, and abundant ground tissue. This organization is consistent with previous descriptions of vegetative anatomy in members of the Amaranthaceae and reflects the structural requirements of rapidly growing herbaceous species **[7,8]**.

The abundance of thin-walled cortical parenchyma indicates that the stem functions not only in mechanical support but also in storage of water and assimilates. Extensive intercellular spaces within the cortex may facilitate internal gaseous diffusion, thereby supporting active metabolic processes in developing tissues. Similar anatomical arrangements have been reported for herbaceous plants characterized by rapid vegetative growth and high physiological activity.

One of the most conspicuous features observed in the stem was the presence of large lignified xylem vessels with wide lumina. Large vessel diameters generally enhance hydraulic conductivity, enabling rapid transport of water and dissolved mineral nutrients from the root system to aerial organs. Thick secondary walls reinforce these vessels against collapse during periods of high transpiration tension while simultaneously increasing mechanical strength. Such hydraulic adaptations are characteristic of fast-growing herbaceous species and contribute to efficient resource acquisition **[13]**.

Supportive sclerenchymatous fibres associated with the vascular bundles further reinforce the stem, enabling it to maintain mechanical stability despite its relatively succulent habit. Together, the combination of lignified vessels and supportive fibres provides an effective balance between flexibility and rigidity, allowing *A. blitum* to tolerate wind, rainfall, and mechanical disturbance.

The occurrence of stomata on the stem epidermis suggests that young stems contribute to gaseous exchange and may participate in photosynthetic activity. Similar observations have been reported in several herbaceous dicotyledons, where green stems supplement leaf photosynthesis, particularly during periods of leaf senescence or environmental stress.

Root Anatomy and Water Transport

The root anatomy revealed a well-organized cortical region composed predominantly of thin-walled parenchyma interspersed with conspicuous intercellular spaces. Such tissues provide both storage capacity and internal aeration, thereby facilitating oxygen diffusion within the root system under conditions of reduced soil aeration. This organization may represent an adaptive advantage in periodically waterlogged or compacted soils where gaseous exchange is restricted.

The vascular cylinder contained large lignified xylem vessels arranged compactly within the conducting tissues. Wide xylem lumina facilitate efficient upward movement of water and mineral nutrients, whereas the thick secondary walls prevent vessel collapse during transpiration. These observations agree with established concepts of xylem structure–function relationships in vascular plants **[13]**.

An important feature documented in this study was the presence of scalariform secondary wall thickenings within longitudinal xylem vessels. Scalariform thickening provides mechanical reinforcement while maintaining flexibility during elongation of developing roots. These wall modifications are frequently associated with efficient hydraulic conductivity in rapidly growing herbaceous plants and therefore represent an important adaptive characteristic of *A. blitum*.

Large parenchymatous storage cells observed within the cortex and vascular tissues indicate an additional capacity for temporary storage of water, carbohydrates, and metabolic reserves. Such storage tissues may enhance resilience during periods of environmental stress and support rapid vegetative regeneration following favourable conditions.

Taxonomic Significance

The combination of anatomical characters observed in the present study provides a suite of reliable diagnostic features for the identification of *Amaranthus blitum*. Individually, characters such as stomatal type, trichome morphology, epidermal cell shape, vascular organization, and xylem vessel architecture possess taxonomic value. Collectively, however, these characters provide substantially greater discriminatory power for distinguishing *A. blitum* from morphologically similar congeners.

The integration of multiple vegetative anatomical characters is particularly valuable in *Amaranthus*, a genus well known for extensive phenotypic plasticity, frequent hybridization, and considerable morphological overlap among species. Consequently, SEM-derived micromorphological data represent an important complementary dataset for classical taxonomy and may be integrated with molecular phylogenetic, palynological, and chemotaxonomic evidence to improve systematic resolution within the genus.

Ecological and Adaptive Significance

The anatomical organization of *Amaranthus blitum* demonstrates a coordinated suite of structural adaptations that enhance ecological performance under tropical environments. The predominance of abaxial stomata, protective trichomes, differentiated cuticular ornamentation, extensive cortical parenchyma, lignified vascular tissues, and efficient conducting elements collectively improve water-use efficiency, mechanical support, storage capacity, and environmental resilience. These characteristics are likely to contribute to the broad ecological amplitude of the species and help explain its successful establishment across a wide range of disturbed habitats, cultivated fields, roadsides, and seasonally dry environments. The observed micromorphological adaptations therefore reflect both phylogenetic inheritance and adaptive responses to environmental selection pressures.

Significance of SEM in Plant Systematics

The present study further demonstrates the value of scanning electron microscopy as a powerful tool in modern plant anatomical research. SEM enables detailed visualization of surface and internal micromorphological characters that are difficult or impossible to resolve using conventional light microscopy. The high spatial resolution achieved through SEM provides access to stable taxonomic characters with minimal environmental influence, thereby improving the reliability of species identification and comparative anatomical investigations.

Future systematic studies within the Amaranthaceae should integrate SEM observations with molecular phylogenetic analyses, morphometric approaches, pollen morphology, phytochemical profiling, and ecological datasets to develop a more comprehensive understanding of species relationships and evolutionary diversification.

The present investigation establishes a comprehensive anatomical baseline for *Amaranthus blitum* by documenting detailed micromorphological characteristics of the leaf, stem, and root using scanning electron microscopy. The diagnostic features identified not only strengthen the taxonomic characterization of the species but also provide insight into its ecological adaptations and functional biology. These findings contribute valuable anatomical evidence for future systematic studies within the Amaranthaceae and highlight the importance of SEM as an indispensable tool for plant taxonomic and evolutionary research.

CONCLUSION

The present study provides a comprehensive scanning electron microscopic (SEM) investigation of the leaf, stem, and root micromorphology of *Amaranthus blitum* L., thereby expanding current knowledge of the vegetative anatomy of this economically and nutritionally important species. The study identified several diagnostic micromorphological features, including predominantly anomocytic stomata, polygonal epidermal cells with undulate to sinuous anticlinal walls, simple non-glandular trichomes, distinct cuticular ornamentation, well-developed collateral vascular bundles, large lignified xylem vessels, and scalariform secondary wall thickenings.

These anatomical characteristics confirm the value of vegetative micromorphology as a reliable source of taxonomic evidence within the family Amaranthaceae. The combination of epidermal, cortical, and vascular characters provides a robust set of diagnostic traits that can improve species identification and complement conventional morphological taxonomy, particularly in taxa where phenotypic plasticity and morphological similarity complicate species delimitation.

Beyond their systematic value, the observed anatomical features demonstrate structural adaptations associated with efficient water transport, transpiration regulation, mechanical support, internal storage, and environmental resilience. The higher stomatal density on the abaxial leaf surface, together with protective trichomes, differentiated cuticular ornamentation, and extensive lignified conducting tissues, illustrates an integrated adaptive strategy that enables *A. blitum* to thrive under diverse tropical environmental conditions.

The findings further demonstrate the effectiveness of scanning electron microscopy as a powerful analytical tool for revealing ultrastructural characters that are not readily resolved using conventional light microscopy. High-resolution visualization of epidermal architecture, vascular organization, and secondary wall modifications provides valuable anatomical evidence for comparative systematics and functional plant biology.

Conclusively, this study establishes a comprehensive micromorphological baseline for *Amaranthus blitum* that will serve as an important reference for future investigations in plant taxonomy, comparative anatomy, ecology, developmental biology, and evolutionary systematics. Future studies integrating SEM observations with molecular phylogenetics, palynology, phytochemistry, morphometrics, and ecological analyses will provide a more comprehensive understanding of species relationships and evolutionary diversification within the genus *Amaranthus*.

ACKNOWLEDGEMENTS

The authors express their sincere appreciation to the staff of the **Microscopy and Microanalysis Unit (MMU), University of KwaZulu-Natal, Pietermaritzburg Campus, South Africa**, for providing access to the scanning electron microscopy facilities and technical assistance during specimen preparation and image acquisition. The authors are also grateful to the **Department of Plant Biology, Faculty of Life Sciences, University of Ilorin, Nigeria**, for supporting plant collection, identification, and herbarium authentication.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

AUTHOR CONTRIBUTIONS

MDA conceived the study, carried out field sampling, prepared specimens for scanning electron microscopy, analysed the micrographs, interpreted the data, and drafted the manuscript. **AAA** supervised the research, contributed to the study design, interpreted the anatomical data, critically revised the manuscript for important intellectual content, and approved the final version for publication. Both authors read and approved the final manuscript.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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