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Preliminary study of leaf structure and histochemistry of Cynodon dactylon

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Abstract

Cynodon dactylon (L.) Pers. has strong adaptability to drought and other stresses. In order to clarify the mechanism of its adaptation to adversity ecolo-gy, this study started with morphology and microstructure, and studied the bo-tanical morphological characteristics, anatomical structure and histochemistry in two different habitats by setting two different habitat conditions of flooding and non-flooding.

Keywords

Cynodon dactylon; Blade; Habitat; Dissect

1. Introduction

Cynodon dactylon (L.) Pers., a low-growing herbaceous plant in the Poaceae family, is widely distributed globally, including China. As a typical C4 plant, it exhibits distinct leaf structural characteristics: undifferentiated palisade and spongy tissues, numerous vascular bundles, and interspersed bulliform cells. These traits are hypothesized to underpin its ecological adaptability, yet their functional relevance under environmental stress remains incompletely understood.

Plant leaves are the primary organs for photosynthesis[1]. The leaf structure of Poaceae plants also consists of three basic parts: epidermis, mesophyll, and veins. The epidermis is composed of long cells, short cells, and stomatal apparatus, with bulliform cells on the upper epidermis; the veins of Poaceae plants are parallel veins, usually containing only one vascular bundle per vein.

2. Materials and Methods

2.1. Experimental materials

The experimental materials were collected in March 2022-2023 by digging out the rhizomes of Cynodon dactylon from the field and planting them in sandy soil (non-flooded control group), while another group was planted in sandy soil and then completely submerged with tap water when the environmental moisture decreased. The plants were flooded every two days for 7 days each time, and then sampled after normal growth for two months after sprouting.

2.2. Experimental methods

2.2.1. Morphological measurements

After collection, C. dactylon was inserted into sandy soil and sprouted after one month. Within two months after sprouting, the length, width, and thickness of the leaves were measured weekly using a tape measure and vernier caliper. At the same time, the number of stem nodes, internode diameter, and internode length of the aboveground part of the plant were measured.

2.2.2. Histochemical and anatomical methods

Hand sections were made using a double-edged razor blade under a stereomicroscope (JNOEC JSZ6, China). Cross-sections were cut for each young and mature leaf, leaf sheath, and stem. Each set of fixed sections (representing each segment distance from the root tip, young and mature rhizome, stem, leaf sheath, and leaf blade) was divided into two groups, with each group containing 3-6 sections from different specimens.

Sudan 7B staining method[2] , Each group of sections was stained with 0.1% (w/v) Sudan Red 7B (SR7B) to test for suberized cell walls (Brundrett et al., 1991) or 0.1% (w/v) berberine bisulfate-aniline blue (BAB) to test for Kearney bands (bright yellow fluorescence) and lignified cell walls (fluorescent dull yellow). Slices were stained with 0.05% (w/v) toluidine blue (TBO) to reveal tissue structure.

Fresh leaf sheath and leaf sections were not stained. All sections were washed 2–3 times with sterile water, fixed in sterile water, and examined under brightfield microscopy using a Leica DME microscope (Germany). Samples were photographed using a digital camera and micrometer (Nikon E5400, Japan). BAB-stained specimens were observed under ultraviolet (UV) light on an Olympus IX71 epifluorescence microscope equipped with an excitation filter G 365 nm, an absorption filter barrier U-WB (blue light), a dichroic mirror DM 500, a compensation excitation filter BP 450–480, and a compensation absorption filter BA 515. BAB-stained specimens were photographed using a digital camera and micrometer (RZ200C-21, Ruizhi Police Bureau, China).

3. Results and Analysis

3.1. Ecological traits of Cynodon dactylon leaves

In two different habitats, the cross-section of C. dactylon leaves consists of epidermis, mesophyll, and veins. The upper and lower epidermis are composed of single-layer cells with different thicknesses and densities, with a large number of bulliform cells in the upper epidermis. The mesophyll structure is very simple, with only one layer of parenchyma cells surrounding the vascular bundles.

The overall morphological characteristics of C. dactylon in non-flooded environment were as follows: within 30 days, the leaf length increased by 122%, the leaf thickness increased by 80%, the leaf width increased by 4.67%, the number of stems increased by 30.13%, the internode diameter of stems increased by 16%, and the internode length of stems and leaves increased by 89%. The C. dactylon in flooded environment; within 30 days, leaf length increased by 56%, leaf thickness increased by 40%, and leaf width increased by 1.67%.

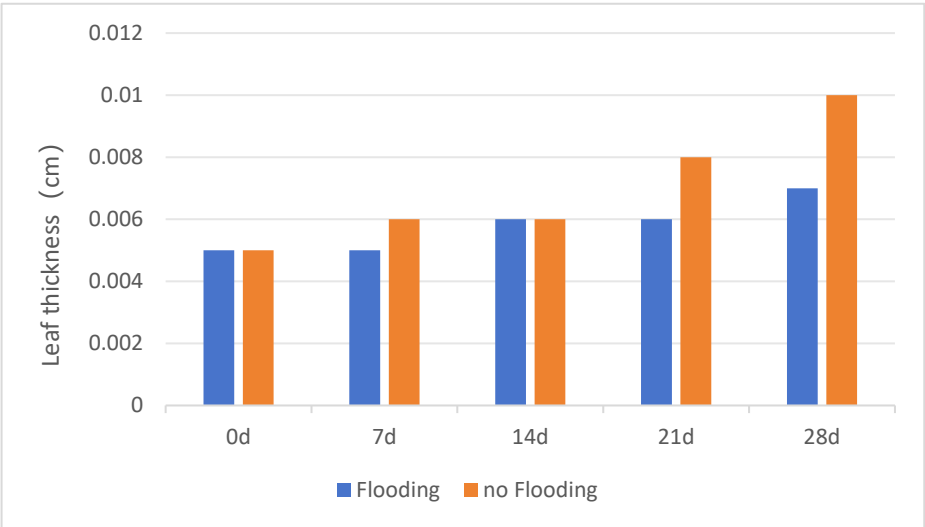

Fig 1. Effects of different habitats on leaf thickness of Cynodon dactylon

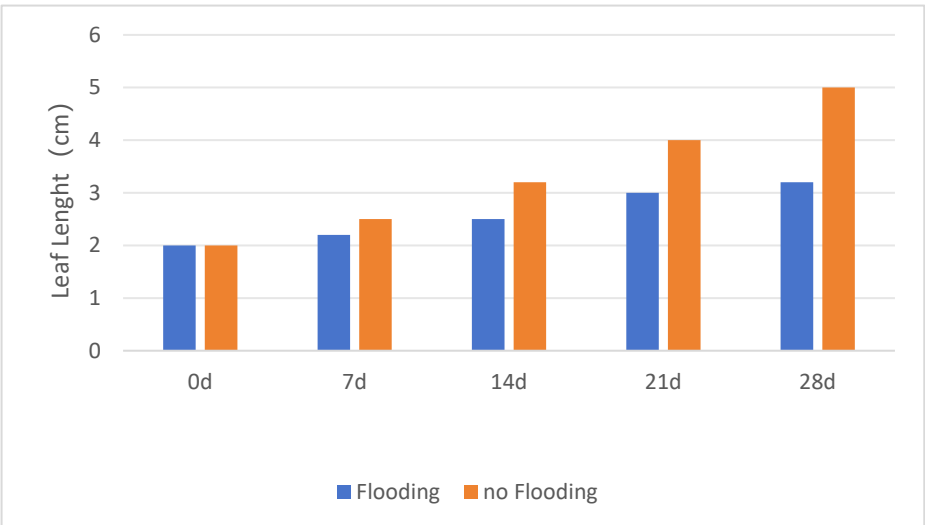

Fig 2. Effects of different habitats on leaf length of Cynodon dactylon

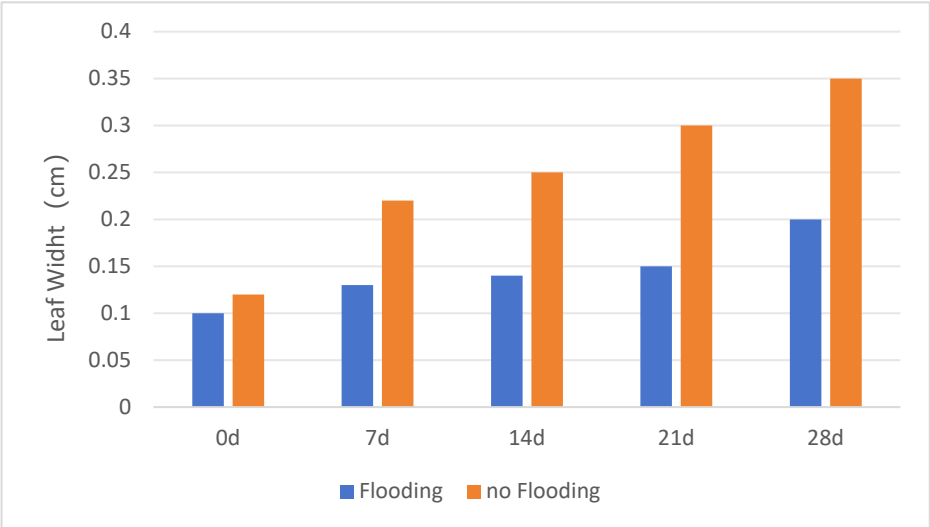

Fig 3. Effects of different habitats on leaf width of Cynodon dactylon

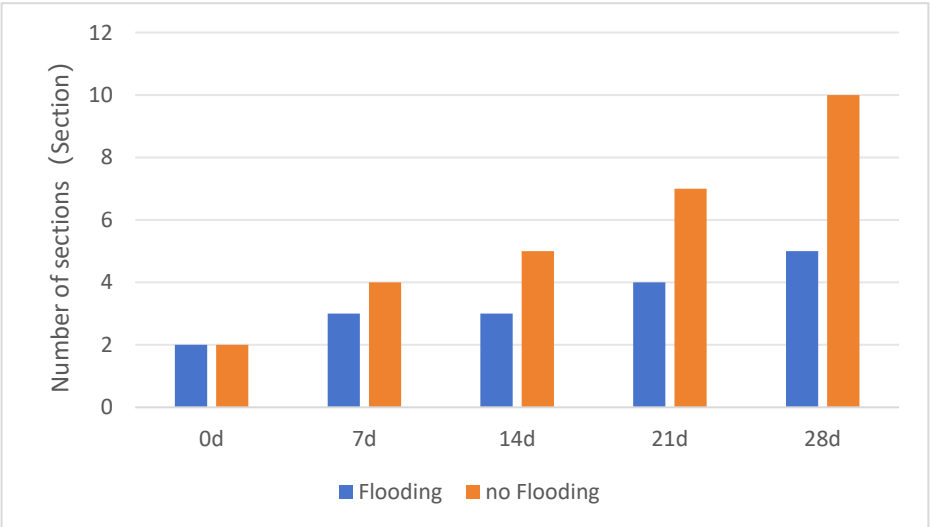

Fig 4. Effects of different habitats on the number of rhizome nodes of Cynodon dactylon

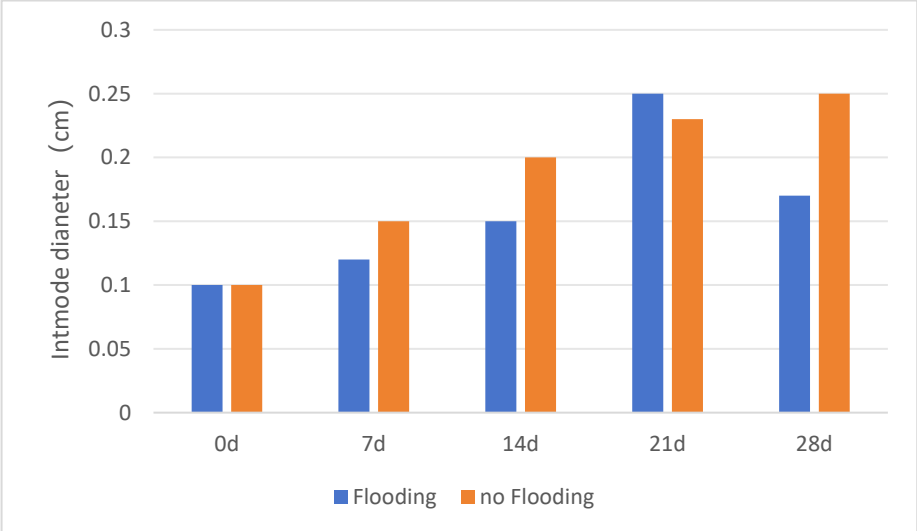

Fig 5. Effects of different habitats on the internode diameter of Cynodon dactylon

3.2. Histochemical and anatomical structure of Cynodon dactylon

C. dactylon normally flowers and grows in June, and the leaves of C. dactylon in flooded environment grow slowly. The experimental results show that the anatomical structure and histochemical characteristics of C. dactylon are similar in non-flooded and flooded environments.

3.2.1. Structure of a Leaf

In cross-section, the leaf blade of Bermuda grass features a central arrangement of large and small vascular bundles, mesophyll tissue, and upper and lower epidermis (Fig. 1A – C). Large vascular bundles possess lignified mesophyll sheaths and parenchyma sheaths, while small vascular bundles contain only parenchyma sheaths, forming a whorl structure (Fig. 1A – C). Both sheath types contain chloroplasts, with cell walls featuring Kearney bands, lignin, and suberin layers (Fig. 1A – C). Lignification occurs in both upper and lower epidermis; subepidermally, globular cells are present. The central vein contains pith, with lignified layers and hypoplastic epidermis on both the adaxial and abaxial surfaces. Lignified sclerenchyma bundles and lateral vascular bundles arranged along the abaxial surface possess sheath layers, while the endodermis contains Köhler's zone, lignin, and suberin (Fig. 1D – E).

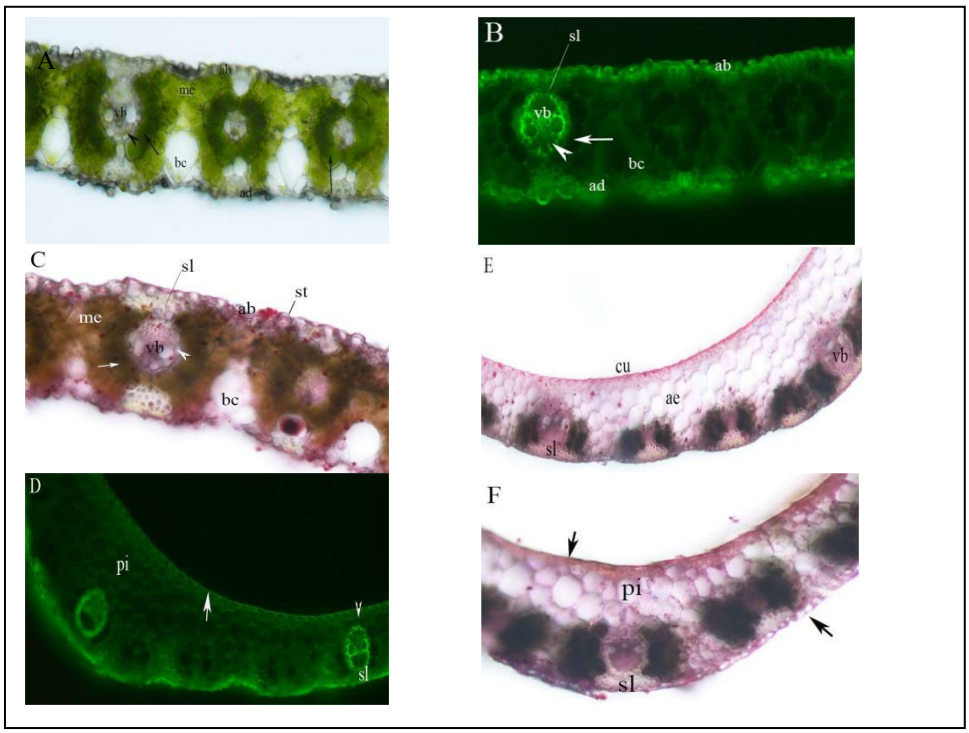

Fig 6. Anatomy of root leaves of a non-flooded Cynodon dactylon

Fig. 7. A–F. Photomicrographs of the leaf blades (A–E) of Cynodon dactylon Scale bars = 50 μm. A. Vascular bundles with mestome sheaths (arrowhead) and parenchymatous sheaths (arrow), mesophyll, bulliform cells, adaxial epidermis, and abaxial epidermis; small vascular bundles to the right have only parenchymatous sheaths (thin arrow). Unstained; B. Vascular bundles with mestome sheaths (arrowhead) and parenchymatous sheaths with Casparian bands (endodermis) (arrow), bulliform cells, suberized adaxial epidermis, suberized abaxial epidermis, and heavily sclerified bundles. Inset shows the Casparian bands (arrowhead) of the parenchymatous sheaths of the small vascular bundles. Staining: BAB; C. Vascular bundles with mestome sheaths (arrowhead) and suberized parenchymatous sheaths (arrow), mesophyll, bulliform cells, stoma, adaxial epidermis with cuticles, abaxial epidermis with cuticles, and heavily sclerified bundles. Staining: SR7B; D.Pith, vascular bundle sheaths with Casparian bands (arrowhead), lignified layer (arrow) under adaxial epidermis, cuticle, and heavily sclerified bundles. Staining: BAB; E. Pith, suberized vascular bundle sheaths (arrowhead), suberized epidermis (arrow), and heavily sclerified bundles. Inset shows an enlargement of the suberized epidermis (arrowhead). Staining: SR7B; F. Vascular bundles, large bundle sheath extensions (arrowhead), aerenchyma, cuticle, and heavily sclerified bundles. Staining: SR7B. Inset shows abaxial epidermis with trichomes (arrowhead). Unstained;

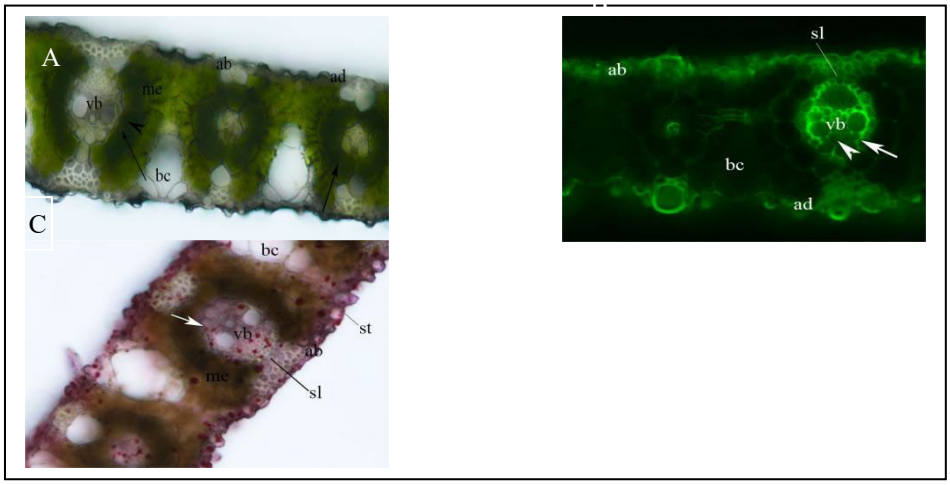

Fig 8. Anatomy of root leaves of a flooded Cynodon dactylon

Fig. 8. A–C. Photomicrographs of the leaf blades (A–E) of Cynodon dactylon Scale bars = 50 μm.A. Vascular bundles with mestome sheaths (arrowhead) and parenchymatous sheaths (arrow), mesophyll, bulliform cells, adaxial epidermis, and abaxial epidermis; small vascular bundles to the right have only parenchymatous sheaths (thin arrow). Unstained; B. Vascular bundles with mestome sheaths (arrowhead) and parenchymatous sheaths with Casparian bands (endodermis) (arrow), bulliform cells, suberized adaxial epidermis, suberized abaxial epidermis, and heavily sclerified bundles. Inset shows the Casparian bands (arrowhead) of the parenchymatous sheaths of the small vascular bundles. Staining: BAB; C. Vascular bundles with mestome sheaths (arrowhead) and suberized parenchymatous sheaths (arrow), mesophyll, bulliform cells, stoma, adaxial epidermis with cuticles, abaxial epidermis with cuticles, and heavily sclerified bundles. Staining: SR7B;

3.2.2. Stem Structure

The central pith of dogtooth grass stems consists of parenchymatous tissue, with vascular bundles scattered throughout several layers of parenchyma extending from the pith to the narrow cortex. The outer surface of young culms features a peripheral ring of sclerenchyma cells and an epidermis (Fig. 2A-C). The vascular bundles possess a multi-layered, suberized and lignified sheath and primary xylem (Fig. 2B-C). Beneath the suberized epidermis lies a single outer epidermis containing the Kessner's zone, lignin, and a cork layer (Fig. 2B-C). The peripheral parenchyma ring contains a small amount of lignin (Fig. 2B).

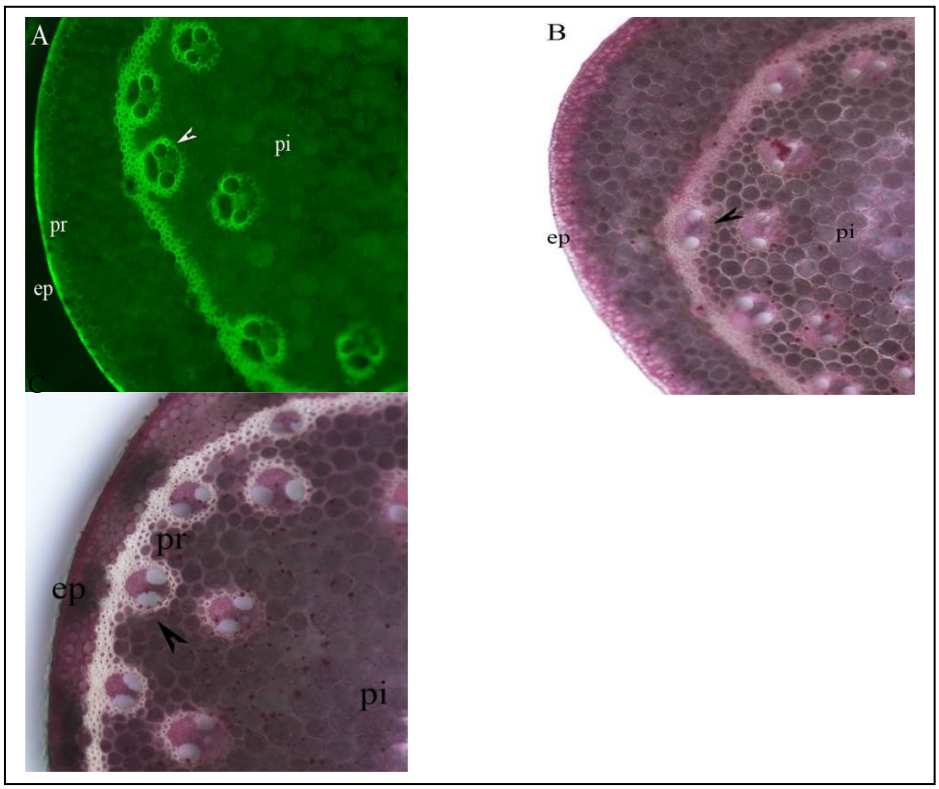

Fig 9. Anatomy of the rhizome of Cynodon dactylon

Fig. 9 A – C. Photomicrographs of the culms of Cynodon dactylon. Scale bars = 50 μm. A.Pith, cortex, lignified vascular bundles with multi-layer sheaths (arrowhead), suberized primary xylem poles (thin arrowhead), lignified peripheral sclerenchyma ring, uniseriate exodermis with Casparian bands (arrow), and suberized epidermis. Inset shows an enlarged view of the lignified peripheral sclerenchyma ring, B.Pith, cortex, suberized vascular bundles with multi-layer sheaths (arrowhead), suberized primary xylem poles (thin arrowhead), suberized exodermis (arrow), and epidermis. Inset shows an enlarged view of the suberized uniseriate exodermis (arrowhead) and epidermis. Staining: SR7B; C. Pith, cortex, suberized vascular bundle sheaths (arrowhead), suberized peripheral sclerenchyma ring, and epidermis. Staining: SR7B;

3.2.3. Structure of Rhizomes

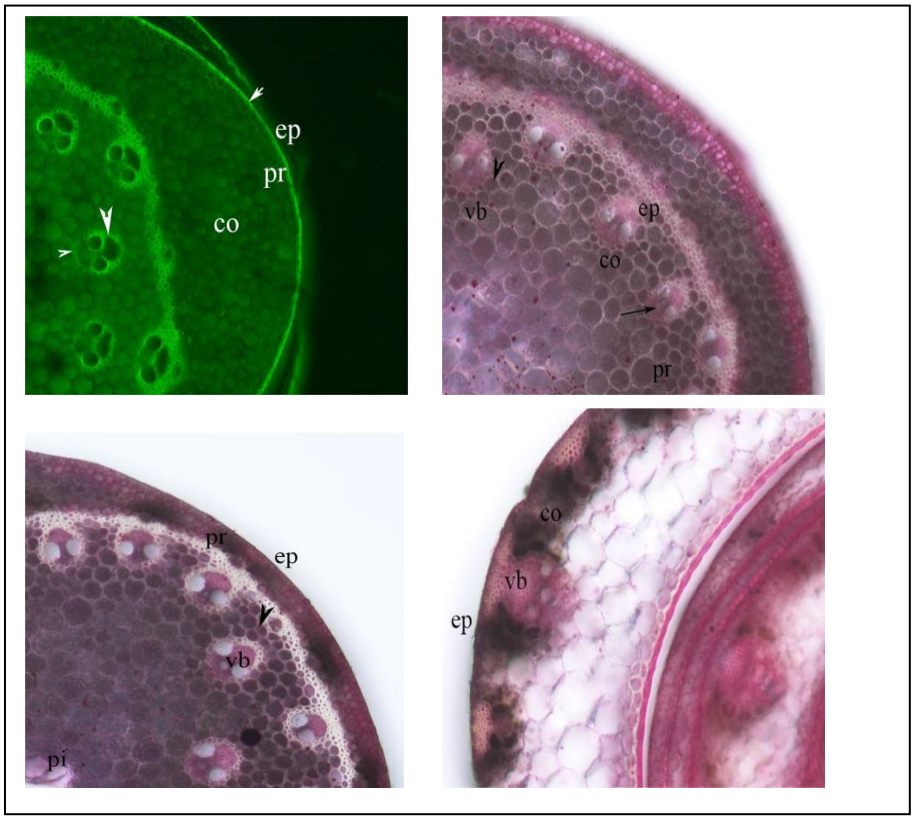

Fig 10. Anatomy of the rhizome of the Cynodon dactylon

Fig. 10. A–D. Photomicrographs of the rhizomes of Cynodon dactylon. Scale bars = 50 μm. A.Marrow, cortex, lignified vascular bundles, with multilayer sheaths (arrows), bolted primary xylem poles (thin arrows), lignified surrounding thick-walled tissue rings, with kjeldahl bands (arrows), and suberized epidermis. Staining : BAB ;B. Pulp cavity, cortex, multi-layered sheath of the bolted vascular bundle (arrow), primary xylem pole (fine arrow), peripheral thick-walled tissue ring, outer epidermis (arrow) and epidermis. Staining : SR7B ; C. The medulla, cortex, aerenchyma, lignified vascular bundle sheath, endodermis with casparian strip (arrow), lignified peripheral sclerenchyma and epidermis. Staining : SR7B ;D.The medulla has cavity, cortex, aerenchyma, embolic joint vascular bundle sheath, endodermis (arrow), embolic peripheral thick-walled tissue ring and epidermis.

Using manual sectioning techniques, we examined the anatomical structure and histochemical characteristics of Bermuda grass under two habitat conditions to analyze its structural adaptations to flooded environments. Results indicate that leaf anatomical structure and histochemical features remain similar between non-flooded and flooded conditions.

4. Discussion

According to the research of Xing Yi et al.[3] populations with similar habitat characteristics have similar phenotypic characteristics. The results of this study on the anatomy of C. dactylon leaves are basically consistent with the observed morphological characteristics, habitat types, and geographical distribution. Using leaf anatomical data for taxonomic studies of Poaceae plants, many species have been classified. It would be better if we could integrate information from multiple aspects such as morphology, anatomy, and molecules.

At present, most studies have shown that C. dactylon can adapt to flooded environments by changing its morphology[4-6] and increasing antioxidant enzyme activity[7-8], and can quickly recover photosynthetic and growth capacity after the removal of flooding stress[9]. In flooded environments, hypoxia is one of the main factors affecting plant growth.[10-12] Prolonged oxygen deficiency will cause plants to gradually shift from aerobic respiration to anaerobic respiration, [13-14]during which the synthesis of ATP and the assimilation of photosynthetic products in plants will be greatly reduced, and plant growth will therefore be significantly affected.[15]

The classic method for studying the Käser zone involves observing the thickening, suberization, and lignification of the radial walls in the root endodermis under a standard optical microscope. It is now established that the Casparian strip comprises Casparian strip membrane proteins, suberization, and lignification, exhibiting polymorphism across species and tissues/organs. Beyond its common presence in root inner and outer periderms, it is also observed in stems, pedicels, and petioles[16-18]. Relying on a single research method often leads to experimental results that prove difficult to interpret satisfactorily.

5. Conclusion

The anatomical structure of C. dactylon leaves is all primary structure, and the characteristics of C. dactylon leaves determine its strong survival ability. In the evolution of plants, as a tissue extremely sensitive to environmental changes, changes in the environment often cause changes in the length, width and thickness of leaves. In different habitats, changes in leaf shape also reflect its adaptability to the environment.

The morphological structure of leaves is the most sensitive to changes in habitat conditions and has great plasticity. Therefore, the leaf structure of plants can truly reflect their characteristics of adapting to the environment. In the early stage of flooding, the growth of C. dactylon leaves in flooded environment was not inhibited, but compared with non-flooded C. dactylon, the growth potential of C. dactylon leaves in flooded environment showed a decreasing trend.

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