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Comparative studies on Leaf and Stem Anatomy of Two Cyperus species

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Abstract

Cyperus rotundus and C. difformis are members of Cyperaceae family which are well-known problematic weeds in the agricultural ecosystem. Although they have different morphological and anatomical characteristics, belong to the same genus and family. The leaves and stems of both species were anatomically characterized in this study to identify diagnostic features and determine a possible relationship between C. rotundus and C. difformis. Epidermal and cross sections of leaves and stems were examined using standard anatomical methods. Transverse sections of leaves and stems of both species displayed useful diagnostic traits. The absence of kranz tissue and minor vascular bundles in C. difformis leaves contrasts with the presence of kranz tissue and minor vascular bundles in C. rotundus. The center of the leaf is occupied by a major vascular bundle encircled by a bundle sheath in both species. The existence of air cavities in the transverse section of C. difformis stem indicated their growing habit under submergence. The higher stomatal density and less interveinal distance were observed in C. difformis than C. rotundus. The results showed that leaf anatomical characters in two species, such as the presence of kranz tissue and interveinal distance, provide a reliable basis for the Cyperus genus, which contains species with both C3 and C4 plants. C. difformis is a C3 species, while C. rotundus is a C4 species.

Keywords: *Oat, Leaves, Nano Urea, Plant height, Fodder quality, Yield and Kent*

Introduction

Cyperaceae is one of the largest monocotyledonous sedge families, because it has a specialized group of vascular plants [1]. *Cyperus* is a large genus with over 600 species is found all over the world [2]. The sedge family has a reputation for being taxonomically challenging, as evidenced by the use of anatomical features of the vegetative organs for taxonomic purposes and the presence of species having both C_3 and C_4 photosynthetic pathways [3]. In India, the dominant species in garden land and wetland are *C. rotundus* and *C. difformis*, respectively. *C. difformis* is a sedge that ranks among Holm's world's worst weeds. It is a troublesome weed, especially in rice and sugarcane fields. It is the dominant one in direct-seeded rice and occurs in high plant densities, forming thick mats of vegetation in the crop at initial stage, resulting in 12-50% yield loss in rice crops. *C. rotundus* is a perennial pestiferous weed, mostly occurs in irrigated uplands, that causes significant yield loss in most crops due to its prolific tuber production and underground rhizome. It also has allelopathic properties, which might affect the growth and development of the crop. The effective control of these weeds is important for obtaining higher agricultural productivity. The first step in determining the best control approaches is to examine the morphology and anatomical features of plant leaves and stems. Understanding the morphology and anatomical features of plant leaves and the stem is of prior importance for realizing the best weed control.

Metcalfe [3], Govindarajalu [4], Rad and Sonboli [5] and Silva *et al.* [6] investigated the anatomy of certain Cyperus species as well as other Cyperaceae genera. Anatomical findings can reveal information on a plant cell type, amount and arrangement, as well as

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its intercellular structure [7] [8]. The leaf and stem anatomical traits of *C. rotundus* and *C. difformis* are yet to be determined. Considering the structural importance for their management, the leaves and stems of both species were anatomically characterized in this study identify to diagnostic features and determine possible phenotypic relationships between *C. rotundus* and *C. difformis.*

Novelty statement

Cyperus rotundus and *C. difformis* are members of Cyperaceae family which are well-known problematic weeds in the agricultural ecosystem. The result shows that anatomical features of the leaf and stem of the studied species, such as the presence of bulliform cells, major vascular bundles and their positions about air cavities, the presence of minor vascular bundles in the leaf, the morphology of leaf blade, stem ground tissues and air cavities, stomatal density, interveinal distance, and *kranz* tissue provides valuable anatomical features that aid in taxonomic delimitation. It should also be mentioned that when all of these features are employed together rather than a single character, the resolution of these traits for species identification is higher. The findings also provide reliable information regarding the *Cyperus* genus, which includes both C_3 and C_4 plant species.

Materials and Methods

The sample of *C. difformis* and *C. rotundus* specimens were obtained from the Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, India from the Wetland Farms and Eastern Block Farms, respectively. The plant was thoroughly cleaned under running water to remove soil and other debris. For this experiment, fully developed leaf and stem segments were collected. The samples were immersed in Formalin-Aceto-Alcohol (FAA), from which sections were made. A sterile blade was used to cut the leaves into thin hand sections. Numerous temporary and permanent sections were made and washed with water. Lactic acid (50%) was used to clear the perfect sections. After that, the sections were stained with safranine (0.1%). Glycerol (10%) was used to mount the sections in the slides. Under compound microscope, the mounted semi-permanent slides were examined and photographed. The qualitative characteristics of the sample were compared in which phenotypic similarity was identified. The terminologies used to describe the anatomy of leaves were adopted from Metcalfe [3], Bruhl [9] and Bugg *et al.* [10].

Stomatal density was calculated as the number of stomata per unit area. Interstomatal distance (average distance between stomata along longitudinal leaf axis) and interveinal distance (distance between vein centers) were measured. A compound microscope was used to observe and capture images of sections. Selected images were imported into ImageJ (1.53e) software (image analyzing software) and all quantitative characters were measured using the software's calibrated micrometer scale. Data from 25 measurements (n=25) were gathered and mean values were reported.

Results

Visual identification of *C. rotundus* and *C. difformis* is not difficult, because the inflorescence of the two species differs. *C. difformis* has dense, globose, umbellate heads with yellowish-brown or pale-brown inflorescence (Fig. 1A). The inflorescence of *C. rotundus* is a compact umbel of spikes that are purplish to red-brown and have a simple and slightly compound appearance (Fig. 1B).

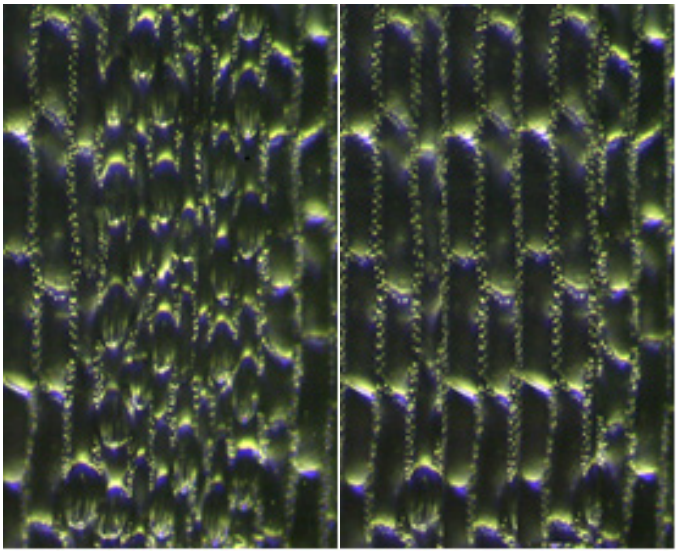
Anatomical features of leaf

Both *C. rotundus* and *C. difformis* come under hypostomatous, with more number of stomata found only on the lower surface of leaves (Fig 1(C-J)). Leaves have epidermal cells on their upper surface. Stomatal density varied widely with *C. difformis* had the highest mean value (19 mm^{-2}) whereas in *C. rotundus* with the lowest mean value (6 mm^{-2}). Inter-stomatal distance differed amongst the species similarly. The mean value of *C. rotundus* and *C. difformis* was 148.7 µm and 46.8 µm. A cross section of the leaf showed that Kranz tissue, which appears as specialized chlorenchymatous leaf bundle sheaths (Fig. 2E), is found in *C. rotundus* species but not in *C. difformis.* In interveinal distance, the mean value of *C. difformis* and *C. rotundus* was 370.7 µm and 93.1 µm (Table 1).

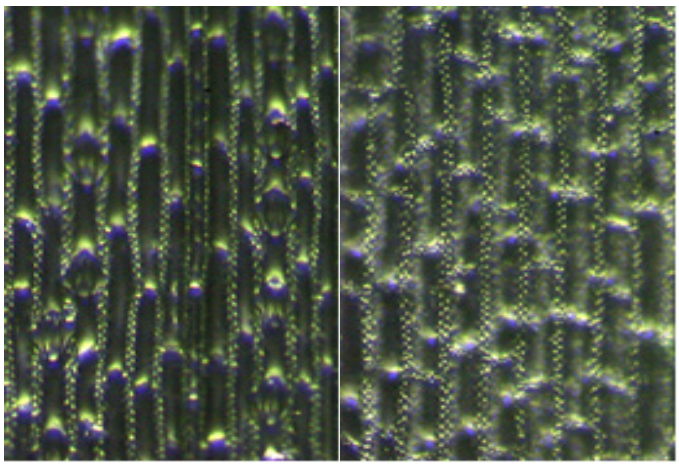
The major morphological difference between the species was that *C. rotundus* had prickle hairs on its leaf blades, but absent in *C. difformis*. Fig. 2 (A-D) and 2 (F-H) shows the transverse section of *C. rotundus* and *C. difformis* leaf, respectively. In terms of the midrib of the leaf foliage, both species have V-shaped flange in trans-section, air cavities and bulliform cells. *C. rotundus* has a flanged V-shaped foliage leaf in trans-section, as well as air cavities and bulliform

A: Cyperus difformis

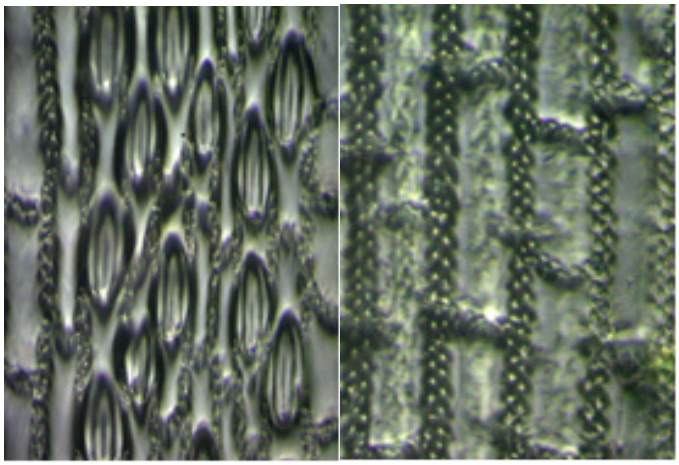
B: Cyperus rotundus



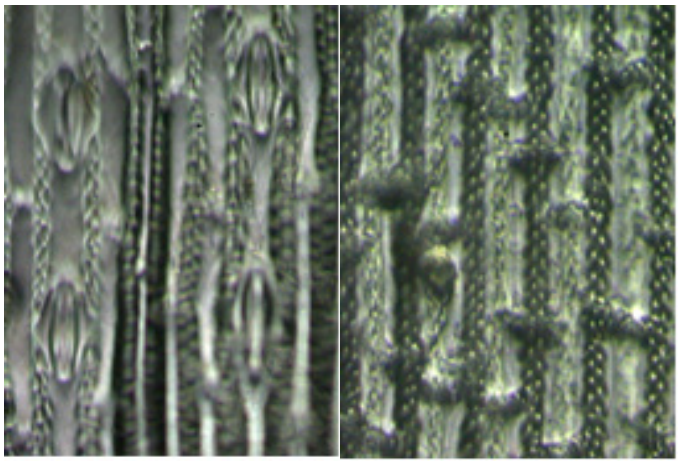
C & D: Lower & upper surface of C. difformis under 10x



E & F: Lower & upper surface of C. rotundus under 10x



G & H: Lower & upper surface of C. difformis under 20x



I & J: Lower & upper surface of C. rotundus under 20x

Fig. 1: Morphological and Microscopic view of upper and lower leaf surface of *Cyperus difformis* and *C. rotundus*

cells on the adaxial side of the midrib. Bulliform cells are single-layered and morphologically dissimilar from epidermal cells in both species. However, the total number of bulliform cells in the leaf differed in both species. *C. difformis* had less bulliform cells (5 nos.) than *C. rotundus* (Table 1).

Table 1: Significant anatomical features of the studied Cyperus species

Features	*Cyperus rotundus*	*Cyperus difformis*
Stomatal density	6 ± 8.46 mm^{-2}	19 ± 17.61 mm^{-2}
Interstomatal distance	148.7 ± 6.10 µm	46.8 ± 11.85 µm
Interveinal distance	93.1 ± 7.56 µm	370.7 ± 14.62 µm
No. of bulliform cells in the leaf	7.1 ± 1.37	4.95 ± 7.43
No. of major vascular bundles	8.5 ± 11.32	12.6 ± 19.23
No. of minor vascular bundles	52.4 ± 7.45	-
Total number of vascular bundles	61 ± 18.77	12.6 ± 19.23
Kranz tissue	Present	Absent
Bulliform cells	Single layered	Single layered
Minor vascular bundles	Present	Absent
Major vascular bundles	Present	Present
Vascular bundles positioned in the leaf	Closer to adaxial surface	Closer to abaxial surface
Air cavities in stem	Absent	Present
Ground tissue of stem	Both parenchymatous and aerenchymatous	Parenchymatous cells
Total No. of vascular bundles in the stem	28.4 ± 11.43	30.2 ± 9.36
Total No. of air cavities in stem	-	27.8 ± 1.48

(Mean ± Standard deviation)

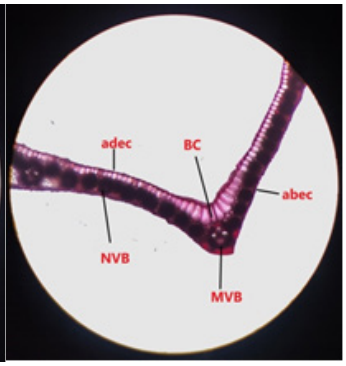
Regarding vascular bundles, both species had major vascular bundles in alternative positions about relation to air cavities. Major vascular bundles were closer to the adaxial surface in *C. rotundus*, whereas they are closer to the abaxial surface in *C. difformis*. *C. rotundus* had minor vascular bundles that were closer to the adaxial surface. In *C. difformis*, minor vascular bundles were absent. Based on the minor and major vascular bundles, the total number of vascular bundles in leaves has differed in both the species. *C. rotundus* had an average of 61 numbers of both major and minor vascular bundles and *C. difformis* had an average of 12.6 numbers of major vascular bundles only (Table 1).

Anatomical features of stem

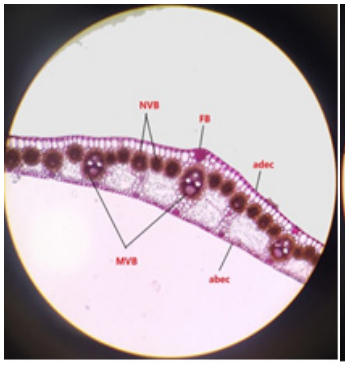
The transverse section of *C. rotundus* stem is shown in Fig. 3 (A-C). In the transverse section, the stem of *C. rotundus* was triangular in shape, sides that were almost flat to slightly concave and grooved with rounded corners. Ground tissue was spongy, breaking down to generate a few big v-shaped cavities. It had thin-walled parenchyma and arenchyma cells which was made up of a large number of collateral and closed vascular bundles. Totally 28.4 vascular bundles were present in the *C. rotundus* stem (Table 1). The vascular bundles present in the outermost circle were smaller in size and embedded in assimilating tissue, and also numerous vascular bundles were distributed in ground tissue. The remaining vascular bundles were larger and scattered in central ground tissue.

The transverse section of *C. difformis* is shown in Fig. 3 (D-F). The stem of *C. difformis* had a star-like shape with three sharp edges. At the periphery, there was a single layer of epidermal cells followed by large air cavities. Various vascular bundles alternately exist between the air cavities (air cavities followed by vascular bundles). *C. difformis* had an average of 30 vascular bundles and 28 air cavities in the stem (Table 1). The spongy tissue in the leaves, roots or stems of aquatic plants that possesses air channels and voids are known as air cavities. Large air cavities present in parenchyma give buoyancy to the plants and allow them to float in water. Each edge had one vascular bundle. The rest of the portion had ground tissue of parenchymatous cells. Both species lacks epidermal hairs (also known as trichomes) and vacuoles.

A: One half portion of C. rotundus leaf

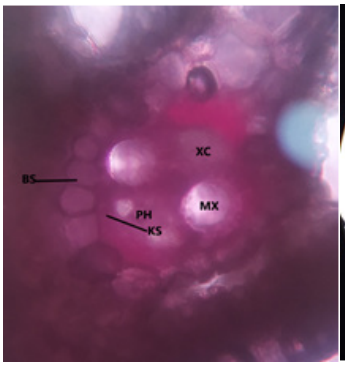


B: Middle portion of C. rotundus leaf



C: Marginal portion of C. rotundus leaf

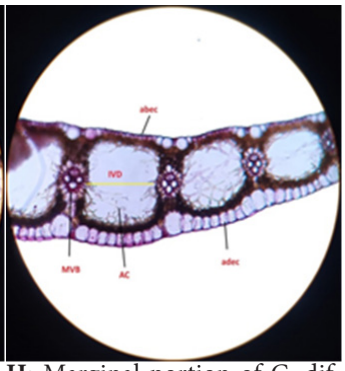
D: Keel portion of C. rotundus leaf marginal



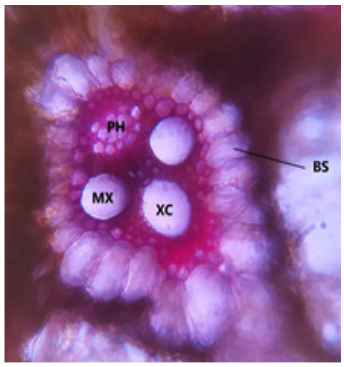
E: Vascular bundle of C. rotundus – closer view

F: Transverse section of C. difformis leaf

G: Middle portion of C. difformis leaf



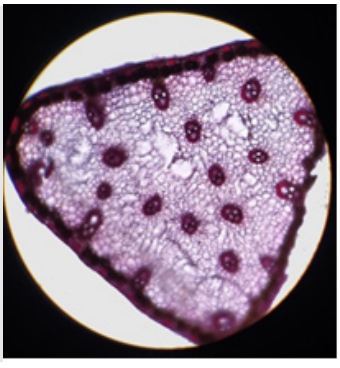
H: Marginal portion of C. difformis leaf



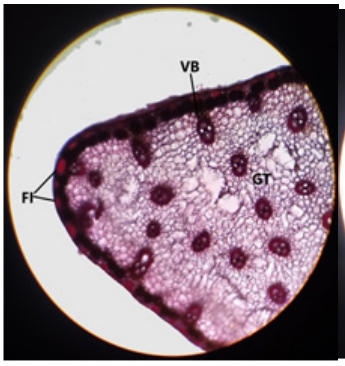
I: Vascular bundle of C. difformis- closer view

Fig. 2: Transverse section of *C. rotundus* and *C. difformis* leaf (bulliform cells (BC), air cavities (AC), adaxial epidermal cell (adec), abaxial epidermal cell (abec), transverse septum between air cavities (TS), major vascular bundle (MVB), minor vascular bundle (NVB), vascular bundles (VB), phloem (PH), metaxylem (MX), xylem cavities (XC), *kranz* sheath (KS)

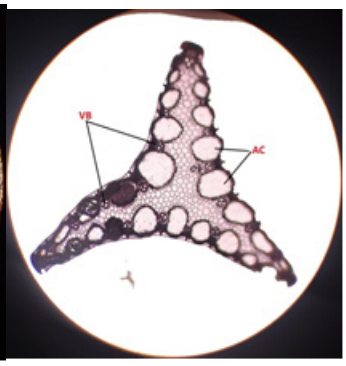
A: Transverse section of C. rotundus stem under 4x



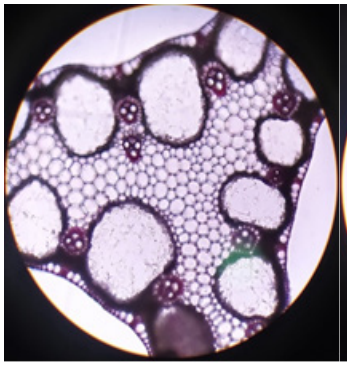
B: Transverse section of C. rotundus stem under 10x



C: Transverse section of C. rotundus stem – half portion



D: Transverse section of C. difformis stem under 4x



C: Middle portion C. difformis stem under 10x

C: One fourth portion C. difformis stem under 10x

Fig. 3: Transverse section of *C. rotundus and C. difformis* stem (air cavities (AC), epidermis (E), vascular bundles (VB), fibre tissue (FI), bundle sheath (BS), ground tissue (GT), phloem (PH), metaxylem (MX), xylem cavities (C)

Discussion

The occurrence of *kranz* tissue, which is associated with C_4 photosynthesis, is a key feature in categorizing *Cyperus* species [11]. This is due to the presence of this structure in C_4 metabolism allows for the spatial dissociation of the photosynthetic enzymes, such as phosphoenolpyruvate-carboxylase (Pepcase), which acts within the mesophyll tissues and Ribulose-1,5-bisphosphate carboxylase (Rubisco) which acts within the vascular bundles [12] [13]. The number of vascular bundles also varied between the species [14] [15] and *C. rotundus* had more vascular bundles than *C. difformis* (Table 1). In the present study, *C. difformis* had no Kranz tissue, implies that it lacks a C_3 photosynthetic pathway [16] [17] [18]. Both species constantly have single-layered epidermal cells and bulliform cells [10] [19]. But the number of bulliform cells varied between species (Table 1). *C. rotundus* had more bulliform cells than *C. difformis*.

Interveinal distances, which are expressed as the average distance between vein centers, is another attribute explored in this study (Table 1). It is widely

assumed that closer vein spacing allows more efficient photosynthates transport between cells [20]. Takeda *et al*. [21] and Li and Jones [22] determined that a species is C_4 if its leaf interveinal distance is less than 130 µm and higher in C_3. Here, *C. difformis* had interveinal distances of 370.7 µm which was more than 130 µm indicates it is as a C_3 species. The other species had an interveinal distance of 93.1 µm, which was less than 130 µm and may be classified as C_4 species [23].

The presence of large air cavities in the transverse section of *C. difformis* stem indicated that it is a semi-aquatic weed that grows under submergence conditions. Sorrell [24] and Silveira *et al.* [25] reported the presence of aerenchyma in the root cortex of *C. alopecuroidesis* as an important anatomical trait of aquatic plants that facilitates their growth and survival in anoxic conditions.

This study showed that a combination of anatomical features can be used as criteria to categorize plant species according to their photosynthetic pathway. In this case, the combination of features, such as the presence of *kranz* tissue and interveinal distance, was shown to give a credible basis for assessing the photosynthetic pathways of the studied *Cyperus* species. The findings support the idea of accurately predicted anatomical data alone may be used to determine the species photosynthetic pathway of Cyperaceae, as suggested by [17]. The anatomical feature from this study reveals that *C. difformis* possess C_3 whereas *C. rotundus* possess C_4 photosynthetic pathways respectively.

Conclusion

Anatomical features of leaf and stem of the studied species, found the presence of bulliform cells, major vascular bundles and their positions about air cavities, the presence of minor vascular bundles in the leaf, the morphology of leaf blade, stem ground tissues and air cavities, stomatal density, interveinal distance, and *kranz* tissue presence provided valuable anatomical features that aid in taxonomic delimitation. When all of these features are employed together rather than a single character, the resolution of these traits for species identification is higher. The findings also result inreliable information regarding the *Cyperus* genus, which includes both C_3 and C_4 plant species.

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Author Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Conflict of Interest

Authors have declared that no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author

Ethics Approval

Not applicable in this paper

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