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SHORT COMMUNICATION

Anatomical investigation of three emergent *Cyperus* species growing naturally on the canal banks of the Nile delta, Egypt

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Abstract

The genus *Cyperus* is a large genus with about 600 species, widespread all over the world. The present work contains anatomical descriptions of culms and leaves anatomy of three taxa of Cyprus spp. (*Cyperus alopecuroides*, *C. articulates* and *C. papyrus*). *Cyperus* spp. were collected from canal banks of Nile Delta. The culms in transverse section of all examined species were triangular except for *C. articulates* which was circular. The ground tissue differentiated into parenchyma cells with thin walls and small, triangular intercellular spaces, while *C. articulates* has large hollow pith. Vascular bundles are small, angular and scattered throughout the thin-walled ground tissue. Leaf anatomy of *C. alopecuroides* and *C. papyrus* is an example of the isobilateral mesophyll with palisade parenchyma on both sides, enlarged epidermal cells referred to as hing cells found in the middle of the blade. The center of the leaf is occupied by a large vascular bundle surrounded by a bundle sheath.

Key words: Sedges; *Cyperus*; aerenchyma; hydrophytes; anatomy

Introduction

Cyperaceae (sedge family) is one among the largest monocotyledonous family and is the third largest family of monocots (Muasya *et al.*, 1998). Sedge family constitute a specialized group of vascular plants with 4000-5000 species and 70-105 genera in the world, particularly in relation to their generative structure (Kukkonen, 1994). *Cyperus* is a large genus with about 600 species, widespread all over the world. In Egypt, three species are distributed in the canal banks of (Boulos, 2005).

It is well known that the sedge family constitutes a taxonomically difficult family and this is reflected when using the anatomical characters of the vegetative organs for taxonomic purposes (Metcalfe, 1971). The anatomy of some *Cyperus* species along with other genera of Cyperaceae has previously been studied by Metcalfe (1971), Govindarajalu (1974), Zarrinkamar *et al.* (2002) and Rad and Sonboli (2008). Anatomical results can provide a view on type, quantity, and arrangement of cells, as well as intercellular structure of a certain plant (Atanackovic *et al.*, 2012; Peng, 2017). Therefore, the objective of our study was to investigate the anatomical characteristics of three *Cyperus* species (*Cyperus alopecuroides*, *C. articulates* and *C. papyrus*) naturally growing on canal bank habitat in Nile Delta, Egypt.

Materials and methods

Collection and fixation of samples

Cyperus alopecuroides and *C. articulates* samples were collected from the canal banks in the Nile delta of Egypt (31°05′46.8″N 31°37′53.4″E), while *C. papyrus* was collected

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from Damietta branch canal bank of the Nile River (31°16′40.3″N 31°41′12.4″E). Samples with 0.5 cm long from fresh materials of leaf and culm of Cyperus spp. were cut and immediately immersed in Formalin-Aceto-Alcohol (FAA) (10:5:85, v\v). The identification of *Cyperus* species was done according to Boulos (2005).

Anatomical investigation

For the anatomical investigation, we followed the methods described by Jensen (1962) and Peacock and Bradbury (1973). The fixed samples were dehydrated in tertiary butyl alcohol (TBA). A series of different concentrations of alcohol (absolute ethanol and in water (50, 70, 85, 95 and 100%) were prepared and the tissues were allowed to remain in each change for 2-4 hours. Then the specimens were kept overnight in TBA for complete dehydration.

For infiltration process, melted soft paraffin wax was poured in vials up to 2/3, and then allowed to solidify. The materials were transferred to the vials a little amount of TBA enough to cover the specimens. Vials were put immediately in an oven at 60°C until TBA evaporates. Then samples were passed through two changes of pure soft paraffin wax, transferred to melted hard paraffin wax and kept in an oven at 60-62°C for two days where the hard paraffin wax replaced the soft paraffin wax.

By using L-shaped metal rods and melted paraplast, a block of wax contained the plant specimen in the middle was prepared. After the block has completely cooled, it was wrapped in a sheet of aluminum foil, and stored in a refrigerator.

Before sectioning, the block was trimmed by removing of excess of paraplast around the specimen. The block was then fixed in a rotary microtome set at the desired section thickness (10-15µm thick). The ribbons were floated in a water bath adjusted at 55°C and then loaded on clean microscopic slides.

Paraffin wax was removed from the sections using two changes of xylene, 20 min. each, followed by the mixture of 1:1 xylene and absolute ethanol for 10 min. each. The sections were dehydrated by passing them through a series of ethanol of decreasing concentration (95, 70, and 50% each). The sections were immersed in fast green stain for 1 min., then washed with water then transferred to safranin for 30 min. The slides were washed with water and dehydrated in different concentrations of ethanol (50 , 70 , 95, 100%) for 1 min. each and clove oil was used to remove the excess stains, then transferred to a mixture of xylene : absolute ethanol (1:1 v/v) for 5 min. Sections were then cleared in 3 changes of xylene and mounted in canada balsam. The slides were placed in an oven set at 30°C for a week to get rid of air bubbles.

Perfect cross sections were selected for examination using full automatic Olympus microscope, photographed at different power and described according to Esau (1977) and Fahn (1982).

Results and discussion

In the present study, leaf and culms anatomical descriptions of *Cyperus* species have been studied. Metcalfe (1971), Rad and Sonboli (2008) and Martins and Alves (2009) reported anatomical characters of some species belonging to this genus (i.e. *C. glomeratus*, *C. malaccensis*, *C. esculentus*, *C. rotundus*, *C. longus* and *C. serotinus*), where our results are in harmony with these studies.

Cyperus alopecuroides

The examined culm was somewhat triangular in transverse section (Figure 1). Epidermis subtended by several layers of assimilatory tissue. The vascular bundles of the outermost circle of the culm are connected to the epidermis by girders of sclerenchyma cells. Ground tissue consists of parenchymatous cells (aerenchyma) with thin walls and small, triangular intercellular spaces, interspersed amongst the cells of the innermost ground tissue. Vascular bundles which are small and angular are scattered throughout the thin-walled ground tissue. The aerenchyma in root cortex is an important anatomical feature of aquatic plants, that supports their growth and survival in anoxic sediments (Sorrell, 1999; Silveira *et al*., 2016).

C. alopecuroides leaf is an example of the isobilateral mesophyll with palisade parenchyma on both adaxial and abaxile sides (Figure 1). Enlarged epidermal cells with thin anticlinal walls, referred to as hing cells found in the middle of the blade, are often described as cells participating in involution and folding movements of leaves. Vascular bundles of different sizes alternate with one another. The center of the leaf is occupied by a large vascular bundle surrounded by a bundle sheath.

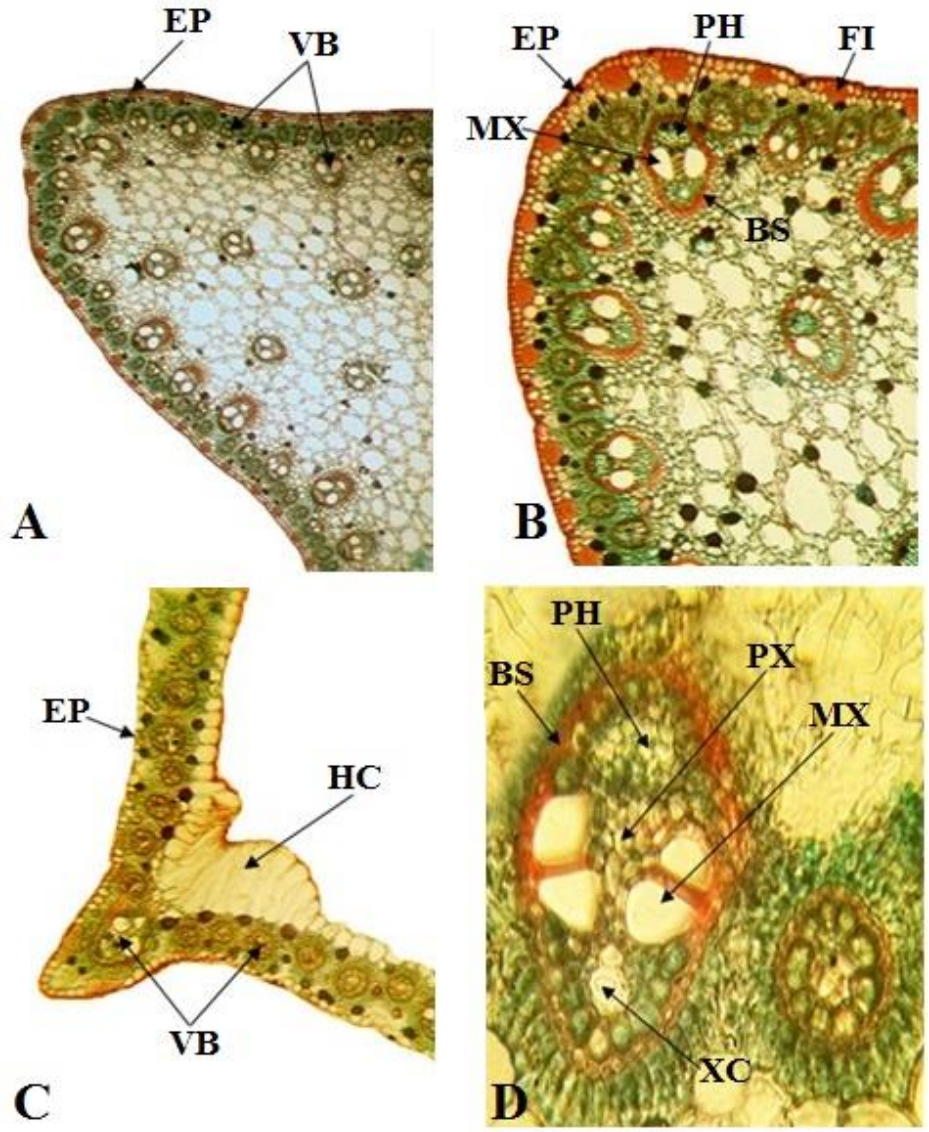

Figure 1. Cross sections of *Cyperus alopecuroides* Rottb. culm and leaf. A, culm; B, enlarged part of culm; C, whole leaf and D, enlarged part of leaf; EP = epidermis; VB = vascular bundle; PC = parenchyma cells; PH = phloem; MX = metaxylem; PX = protoxylem; XC = xylem cavity; BS = bundle sheath; FI = fibres; HC = hing cells.

Cyperus articulates

The circumstance of the culm is more or less circular (Figure 2). The epidermis is uniseriate and subtended by several layers of assimilating cells. The ground tissue differentiated into parenchyma cells and large hollow pith. The vascular bundles are collateral and closed. They have two narrow metaxylem elements and small protoxylem elements and surrounded by a sheath of fibers. The outermost vascular bundles are smaller in size and embedded in assimilating chlorenchyma while, the remaining vascular bundles are large in size and scattered throughout the thin-walled ground tissue.

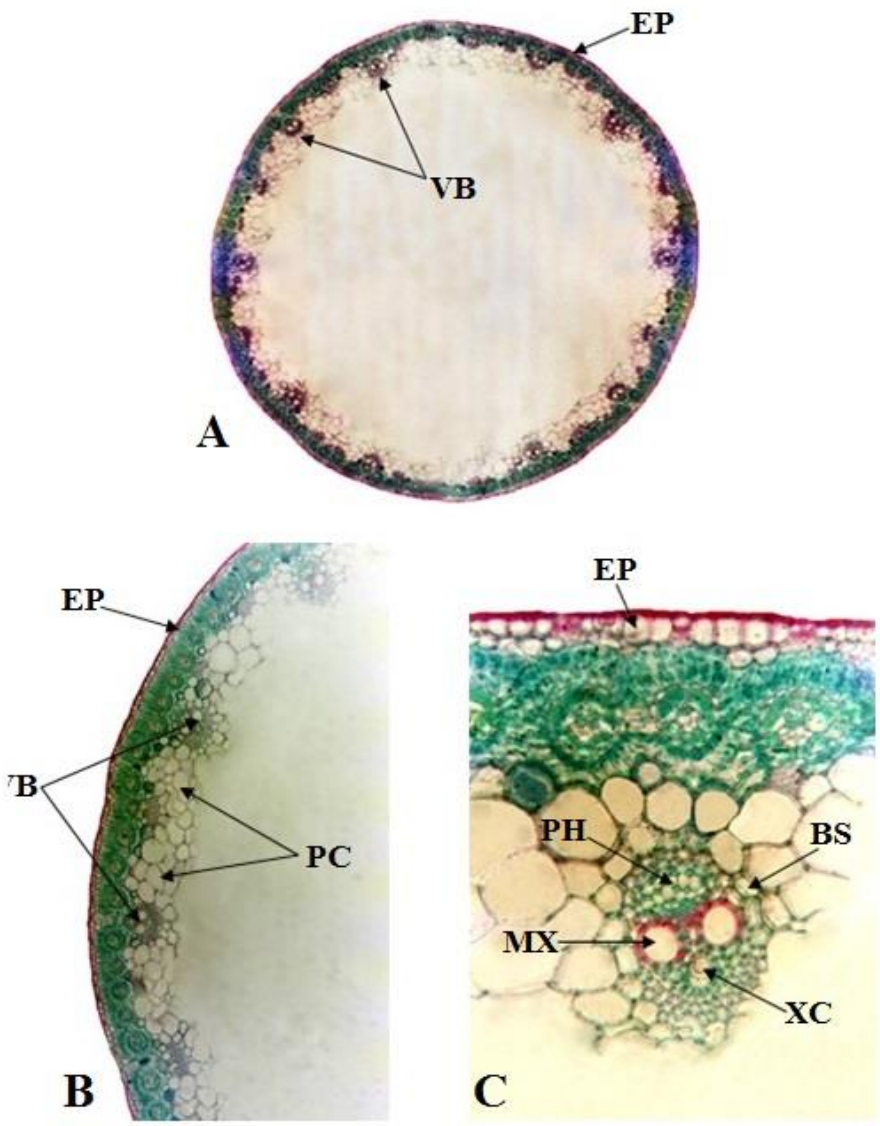

Figure 2. Cross sections of *Cyperus articulatus* L. culm. A, culm; B, part of culm and C enlarged part of culm showing vascular bundle; EP = epidermis; VB = vascular bundle; PC = parenchyma cells; PH = phloem; MX = metaxylem; XC = xylem cavity; BS = bundle sheath.

Cyperus papyrus

Culm is somewhat triangular in transverse section (Figure 3). The epidermis is thick and subtended by ridged sclerenchyma tissue. Several layers of assimilatory tissue found below the sclerenchyma. Ground tissue consists of aerenchyma cells with thin walls and small, triangular intercellular spaces, interspersed amongst the cells of the innermost ground tissue. The vascular bundles of the outermost circle are small in size and embedded in assimilating tissue. The remaining vascular bundles are larger in size and scattered throughout the thin-walled ground tissue.

Cyperus papyrus leaf contained palisade tissue on the adaxial side while spongy tissue consists of large cells found beyond abaxile side (Figure 3). Hing cells are often described as cells participating in involution and folding movements of leaves and it found in the middle of the blade. Vascular bundles of different sizes alternate with one another. The center of the leaf is occupied by a large vascular bundle surrounded by a bundle sheath.

It is obvious that leaves in outline were usually V-shaped (flanged or not), leaf contained palisade tissue on the adaxial side while spongy tissue found beyond abaxile side. According to Metcalfe (1971), expansion or contraction of Bulliform cells is responsible for the rolling and unrolling or for folding and unfolding of leaves.

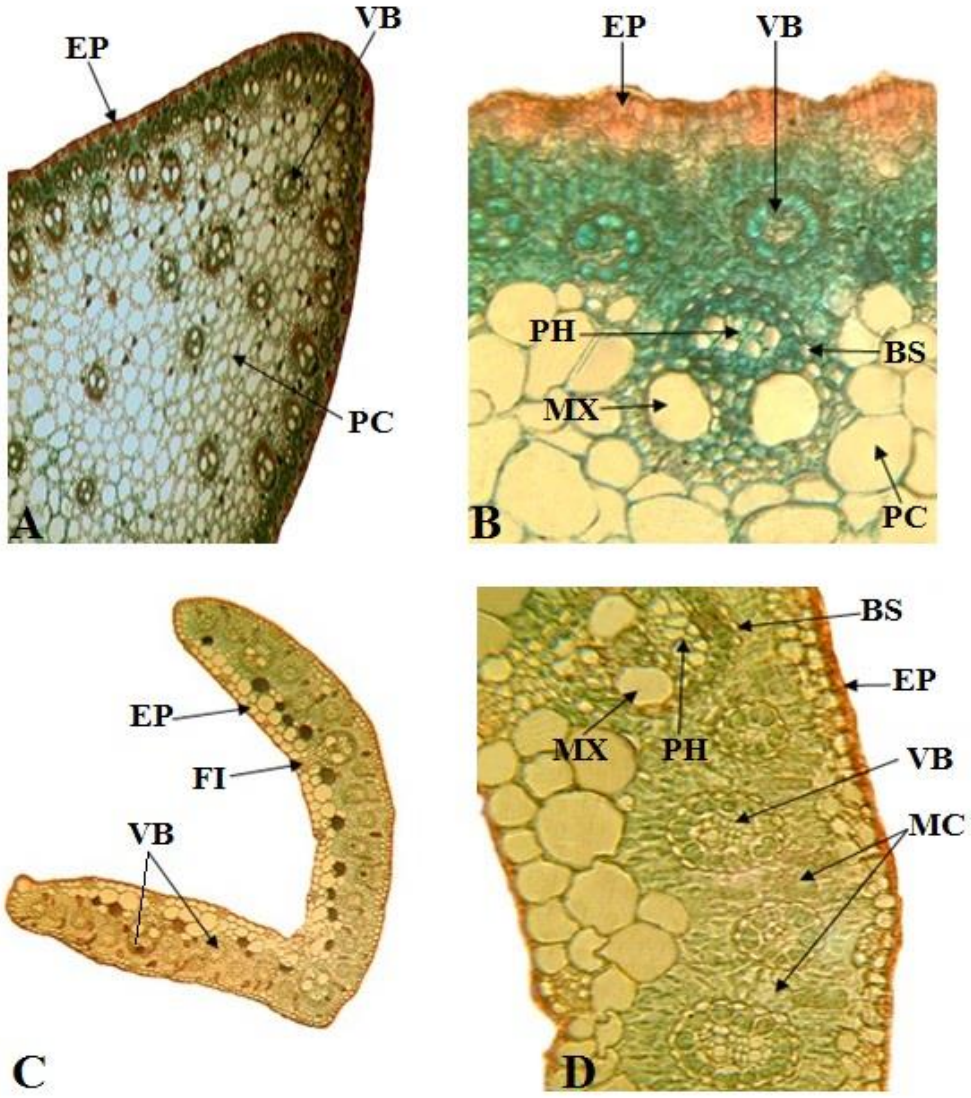

Figure 3. Cross sections of *Cyperus papyrus* L. culm and leaf. A, culm; B, enlarged part of culm showing vascular bundle; C, whole leaf and D, enlarged part of leaf; EP = epidermis; VB = vascular bundle; PC = parenchyma cells; PH = phloem; MX = metaxylem; XC = xylem cavity; BS = bundle sheath; FI = fibres; MC = mesophyll cell.

Bulliform cells are structural features found especially on species included in Poaceae, Cyperaceae, and Juncaceae (Grigore and Toma, 2017).

Cyperus spp. have primary meristems alone and lack true secondary growth. Leaves in outline were usually V-shaped due to expansion or contraction of bulliform cells. Vascular bundles of different sizes alternate with one another. The center of the leaf is occupied by a large vascular bundle surrounded by a bundle sheath.

Author contributions

Ahmed Abd El-Gawad and Yasser El-Amier contributed equally for field work, samples preparation, and manuscript writing. Both authors approved the final version of the manuscript for publication.

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