EFFECT OF DIFFERENT SOILS ON THE STEM ANATOMY OF CYPERUS SPECIES FROM DIFFERENT AREAS OF PUNJAB PAKISTAN

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خلاصہ

پودوں میں پائی جانے والی تبدیلیوں کا انحصار اندرونی وبیرونی عوامل پر پایا جاتا ہے۔ مندرجہ ذیل تحقیق سائپرس کے تنے پر کی گئی ہے۔ پنجاب میں پائے جانے والے اس پودے کے تنے کے مختلف حصوں کا بغور جائزہ لیا گیا۔ مقامی حالات سے جڑے تنے کی اندرونی حصوں میں جو تبدیلیاں رونما کی انکی تفصیل اکٹھی کی گئی۔ اور یہ بات نوٹ کی گئی کہ تنے کی نشوونما کا انحصار زیادہ تر مقامی حالات پر ہوتا ہے

Abstract

After Poaceae and Orchidaceae, Cyperaceae is the third largest family of monocots. It is also called the Sedge-family. Plants of this family are tolerant to different environmental stresses as they can grow in wide range of environmental conditions. Its largest genera include Carex and Cyperus. Cyperus containing the 600 species of sedges. Approximately, 22 genera and 179 species have been reported to be found in Pakistan. Plants of family Cyperaceae are found in a variety of habitat types such as high altitude, steep slopes, extreme aridity, swamps, extreme salinity, moist areas and marshes. *Cyperus* is the largest genus of Cyperaceae family. The transverse sections of stems of different species of Cyperus collected from different areas of Punjab including desert and semi-desert, were cut. The stem sections and soil samples were investigated to analyze the adaptations in stem anatomy with respect to changes in soil parameters. The results showed diverse modifications in the stem area, vascular bundle thickness, phloem cell area, metaxylem cell area, collenchyma cell area, sclerenchyma thickness, and epidermal cell area in different plants depending on the type of soil of the particular region. The modifications were parallel to increased growth rate of the plant in that region.
Keywords: Cyperaceae, Cyperus, Sedge, Stem, Soil.

Introduction

The third largest family of monocots, *Cyperaceae,* is classified into more than 70 genera comprising of around 4000 species. It is among the top ten families in angiosperms and occupies second position in order poales. Moreover, it is a large family of vascular plants with genera ranging between 70-105 and the number of species between 4000-5000. Cyperaceae is also the second largest C_4 family with its 1500 species of C_4 plants that makes up more than 20% of the whole family. However, all the C_4 species of Cyperaceae are very diverse in their biochemical and anatomical features. This proves the varying nature of the C_4 character of angiosperm plants (Besnard et al., 2009).

The participants of Cyperaceae have adaxial epidermal cells longer than the abaxial epidermal cells. Most taxa of the family have very solid bulliform cells in the midrib. Collateral vascular bundles and double-layered bundle sheath having parenchymatous outer layer and sclerified and fibrous inner layer are another distinct characteristic of the family. Many species of the Cyperaceae family also have phytoliths which are the silica bodies in epidermis. Paracytic stomata having two subsidiary cells near to stomatal aperture is also present in some species. Its distribution is cosmopolitan (Metcalfe, 1969).

Cyperaceae is also known as the sedge-family because of its genus *Cyperus. L.* comprising of about 600 species of sedges, ranging all over the tropical and temperate zones. The sedges are aquatic annual/perennial plants growing up to 0.5 cm in water. The sedge-family contains synapomorphy because of the division of three androspores in meiosis (Escudero et al., 2012). The *Cyperus* genus is subdivided into two genera based on the anatomical development. One subgenus is the C_4 type photosynthetic Cyperus that possess Kranz cells with chlorocyperoid anatomy. The second subtype is the C_3 photosynthetic without kranz cells (eucyperoid anatomy) (Carolin et al., 1977). Spikelets are also the distinct feature of Cyperus while the anosporum have digitately arranged spikelets (Holttum, 1948). The morphological characters of the genera such as the spiral arrangement of legumes, pleiomorphic trimerous and laterally and dorsoventrally flattened dimerous gynoecium etc. have already been analyzed using anatomical and ontogenetical techniques (Blaser, 1941).

Due to its diverse nature, the Cyperaceae family is largely distributed all over Pakistan ranging from plains to sandy mountainous areas of Sindh, Punjab, Baluchistan, Gilgit Baltistan, Kashmir and Khyber Pakhtunkhwa (Butt et al., 2018; Marwat, 2008). Studies have reported 22 genera and 179 species in Pakistan so far (Ikram et al., 2014). In Pakistan, Punjab province lies 69°18' to 75°23' east longitudes and 27°42' to 34°02 north latitude and is bordered by Sindh on the South and Khyber Pakhtunkhwa on the west and Baluchistan on the North. The topographical zone covers around 20.63 million hectares while it spreads 1078 km in length from north to south and 616 km in width from east to west.

The soils of different districts of Punjab have different texture and minerals that results in variety of plant growth and distribution of species throughout the region (Condom et al., 1999). For example, district Chakwal has soil made from limestone, sandstone and shale rocks. It is mainly calcareous and weak in structure. The vastly eroded unleveled lands of the district also comprise of materials like rocks, stones and gravels etc. The soil ranges in consistency from sandy loam to loam and has a pH of 7.7-7.8. Another district Murree has two different regions with respect to soil structure. The upper region has reddish or purplish sandstones in the soil while the soil of lower region comprises of greyish sandstones. However, the Murree district is rich in diverse plant species ranging up to 700 in number. The Sahianwala region of Punjab has three different types of soils including dry saline soil, wetland and highly saline soil. On the other hand, the Head Rasool district has dry soils with water shortage. The soil of Faisalabad is Saline-sodic while the soil of Chiniot region is sandy in structure. Kalar kahar is another region of Punjab with saline soil (Hassan et al., 2016).

Soil structure affects the growth and anatomy of various parts of the plant. Comparing anatomical structures of plants are of great significance in determining taxonomy, ecology and diversity of species. This study was designed to evaluate the impact of soil of various districts of Punjab on the stem anatomy of selected species of *Cyperus*. It also aimed to identify the habitat ecology and species diversity of *Cyperus* in various regions of Punjab.

Material and Method

To analyze the soil types of different districts of Punjab, detailed surveys were performed in various cities of Punjab such as Sahianwala, Faisalabad, Sargodha, Rawalpindi, Murree, Nowshera, the Salt Range, Khushab, Lahore, Jhang, Mianwali and cities in some other districts of the Punjab region. Sampling of soil was conducted to analyze the electrical conductivity and concentration of various ions such as K^+, Na^+, Cl^-, and Ca^{2+}. Soil samples obtained from different ecological zones were chemically analyzed in laboratory.

Sahianwala region had three types of soils i.e., dry saline soil, wetland, and highly saline soil. Conductivity meter was used to measure the electrical conductivity (EC). Compound microscope was used to observe the anatomical parameters through an ocular micrometer and calibrated stage micrometer.

Materials were fixed by formalin acetic acid solution (FAA) which contains 5% formalin, 10% acetic acid, 35% distilled water and 50% ethyl alcohol. For long term preservation, acetic alcohol solution (containing 75% ethyl alcohol and 25% acetic acid) was used.

Transverse sections of the stem samples were stained with double-staining standard technique to prepare slides. For the calculation of area of different tissues and cells, following formula modified from the area of a circle was used.

$$\text{Area} = \frac{\text{Maximum length x Maximum width}}{28} \times 22$$

Statistical analysis

Data collected was analyzed using ANOVA (Analysis of Variance) and complete randomized study design with double-factor factorial arrangement was applied.

Results and Discussion

Soil samples and stem anatomy of various regions of Punjab showed that samples of *Cyperus laevigatus* collected from the dry saline and highly saline soils of Sahianwala had the highest stem area and phloem cell area while the *laevigatus* growing in the dry saline soil and wetlands also showed maximum phloem cell area. Similarly, in the head Rasool region, *Cyperus glaber* showed largest cortical cell area, vascular bundle area, metaxylem area, maximum cuticle thickness and second largest epidermal cell area. Another species *C. squarrosus* in head Rasool region had maximum aerenchyma cell area. Likewise, *Scirpus maritimus* collected from Khabeki Lake showed maximum epidermal thickness, thickest sclerenchyma and minimum phloem area. The *Cyperus compressus* growing in the soils of Kalar Kahar showed thinnest epidermis with minimum epidermal cell area and minimum cortical cell area. The analysis of stem anatomy of *C. haspans* collected from

Chiniot demonstrated thick sclerenchyma and thin collenchyma. Another species *C. difformis* in the soils of Jaranwala region of Punjab had thickest collenchyma, thin sclerenchyma and maximum epidermal cell area. *Cyperus iria* collected from Gujranwala had minimum stem area while *Cyperus longus* sections collected from Treemu showed minimum vascular bundle area and *Cyperus alopecuroides* samples obtained from balloki had thinnest metaxylem area in their stem anatomy. Another species *C. rotundus* collected from University of Agriculture, Faisalabad showed minimum aerenchyma cell area in the stem anatomy analysis. All other species had moderate growth rate in these soil regions of Punjab.

Cyperaceae is known to be among one of the core eudicot group. Although Cyperaceae has great, morphological diversity, the family is strong as long with morphological and chemical assessments. Cyperus family is diverse in its distribution. The family occupied a great variety of habitat ranging from temperate forests, damp, marshes, in arctic tundra, in old fields and steep slopes (Ueno & Takeda, 1992; Weber, 2005).

The wide variety of soil structure in different regions of Punjab supports different growth rate of specific species in particular districts of the province. This is due to the adaptation of plants to survive in different climate and soil conditions. The modifications in stem anatomy involve changes in different specialized tissues to cope with the challenges of harsh conditions like salinity and drought (Korn, 2016; Nawaz et al., 2014).

For example, thicker epidermis is a modification for water restricted soils. Studies have also reported that larger cortical cell area is a modification for moisture storage and helps to avoid harsh dry climates as it. So, it is also needed for survival in dry soils. Similarly, vascular bundle area is needed for effective translocation of water and nutrients from the soil to the leaves. Therefore, in all types of soils, suitable modifications of vascular bundle area are also significant for meeting the water requirements of a plant (Nawaz et al., 2014; Pezeshki, 2001).

The efficient growth of *Cyperus laevigatus* in the dry saline and highly saline soils of Sahianwala is attributed to highest stem area and maximum phloem cell area while the wetlands of Sahianwala also have a larger number of the species due to larger phloem area.

The Head Rasool region of Punjab depicted a wide dispersal of *C. glaber*, and *C. squarrosus* species. This is because of multiple modifications in stem anatomy suitable for a variety of climates and soil conditions. Largest cortical cell area, maximum metaxylem area, larger vascular bundle area, thick cuticle, wide aerenchyma cell area and second largest epidermal cell area are the significant features of these species to transport maximum water from the dry soil of Head Rasool region and prevent water loss from the stem.

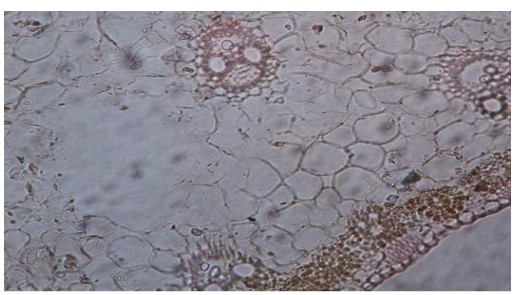
The saline soil of Kalar kahar region has dominance of *C. compressus* species that has stem anatomy with thinnest epidermis and minimum epidermal cell area and cortical cell area. The stem anatomy of all Cyperus species in saline soil show modification to cope with the high salt concentrations in soil. The thinner epidermis evaporates some water from the stem to create a concentration gradient in order to avoid massive water loss in the salty soil. Other Cyperus plants lacking these stem modifications show moderate growth in this region of Punjab. The saline-sodic soil of Faisalabad has acidic pH in the presence of silica while alkaline pH in the presence of limestone. It has the highest percentage of saturation and electrical conductivity. So, in this area there is a dominance of *C. rotundus* with minimum aerenchyma cell area in the stem. The minimum aerenchyma cell area is a modification of the plants of this species to avoid harsh soil salinities.

The sandy habitat of the Chiniot region has a dominance of *C. haspans* species which are more drought-resistant due to their thick sclerenchyma and thin collenchyma in the stem. The large Sclerenchyma is needed to prevent water loss from the stem which help in survival during a water shortage.

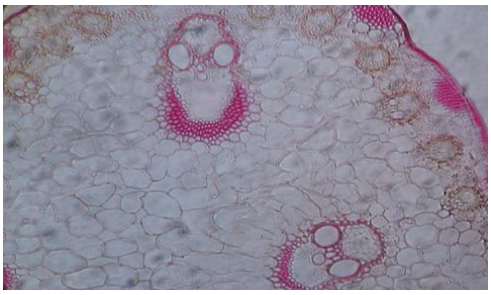
Other species growing in the regions of Jarhanwala, Treemu and balloki also showed specific modifications in the stem anatomy depending on the soil type of the area. This shows that plants undergo important adaptations in their stem anatomy for competing with other species in the same region. Those plants who do not have particular survival modifications show moderate growth in different regions of the same area.

Table 2. Stem anatomical characteristics of some species of family Cyperaceae from the Punjab region

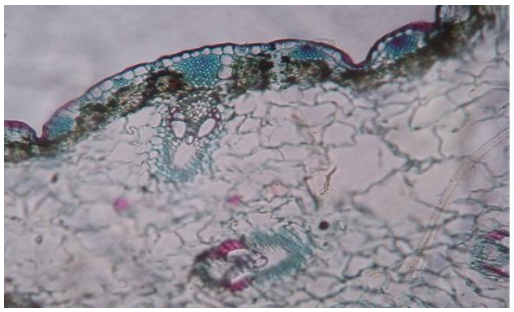
Stem Anatomical Parameters	***C. glaber***	***C. diffformis***	***C. squarossus***	***C. iria***	***C. haspans***	***C. longus***	***C.L wet***	***C.L dry saline***	***C. L highly saline***	***C. nutans***	***C. alopecuroides***	***C. rotundus***	***C. compressus***	***C. alternifolia***	***C. esculuntus***	***Scirpus maritimus***
Leaf area (µm2)	260902.456	295987.70	306565.80	299773.3717	64301.84	127679.9092	83664.85	105074.6423	125433.9	230490.4	191293.4	94641.44	81962.21	61965.24	329460.9	818490
Epidermis thickness (µm)	194.13	13.86	26.34	26.34	11.09	7.06	0.63	0.6	12.68	30.5	29.12	8.32	6.93	8.32	10.4	353.2
Epidermis cell area (µm2)	1394.71	688.30	2372.82	2517.73	434.71	525.28	2626.41	1992.45	1793.2	3115.46	3350.94	217.35	163.01	217.35	108.67	353.2
Lower Epidermis cell area (µm2)	1394.71	688.30	2372.82	2282.26	434.71	398.49	615.84	815.09	1702.64	3115.46	3441.5	815.09	543.39	579.66	163.03	869.43
Upper Epidermis Cell Area (µm^2)	3006.79	16301.88	1557.73	2644.52	344.15	398.49	2264.14	1702.64	2209.81	1938.11	2553.9	869.43	326.03	1919.99	2064.9	271.69
Cortical Cell Area (µm^2)	5130.69	475.90	294.60	942.74	326.33	843.02	1065.11	498.56	339.93	353.52	380.72	135.97	738.78	1110.44	1722.31	1529.69
Lower Epidermis Stomatal Cell Area (µm^2)	299.13			299.13			870.22	1137.63	865.69		951.8	398.85	380.72	552.95	920.08	285.54
Upper Stomatal Cell Area (µm^2)	516.69			516.69			290.07	729.71	294.6		521.22	217.55	262.88	462.3	376.19	278.74
Lower Epidermis Stomatal Number	93.33			52			62	96.66	55.66	33.33	88.33	292.07	98	84	88.66	85
Upper Epidermis Stomatal Number	90.66			86.33			121.33	120.66	77.33	36	105.33	109.33	121.33	102.66	105.33	124
Bulliform cell area (µm2)					2674.12	1495.69				2506.42		1486.63	108.77			
Sclerenchyma Thickness (µm)								72.1				24.96	12.48	12.48		31.2
Collenchyma Thickness (µm)		45.76	87.36	69.33	66.56	83.2				90.13		29.12	34.66	63.78	41.6	72.8
Aerenchyma Cell Area (µm^2)		21483.67	25789.46	8747.56		13252.79	13030.7	4623.06	10914.07	12763.29	8158.35				12273.79	25834.79
Vascular Bundle Cell Area (µm^2)	3095.64	6087.03	4192.48	3353.99	2374.98	2642.4	2379.52	3761.9	2515.49	1767.64	2152.89	4804.36	2402.18	4419.1	2855.42	1808.43
Metaxylem Cell Area (µm^2)	135.97	815.83	135.97	90.64	122.37	330.86	108.77	90.64	321.8	49.85	131.44	281.01	244.75	163.16	385.25	489.5
Phleom Cell Area (µm^2)	1314.4	49.85	77.05	412.45	31.72	421.51	915.54	453.24	462.3	45.32	36.25	235.68	299.13	244.75	462.3	81.58



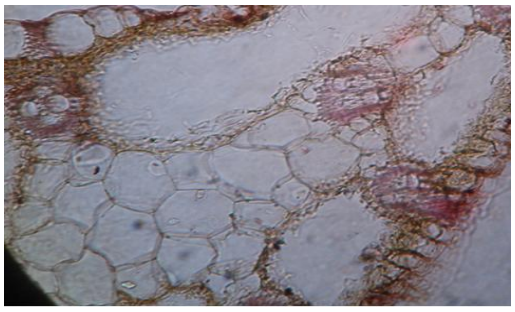
Cyperus haspans



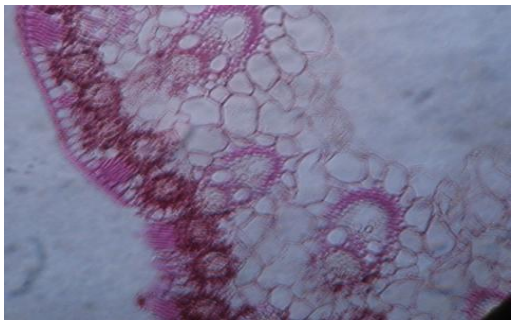
Cyperus longus



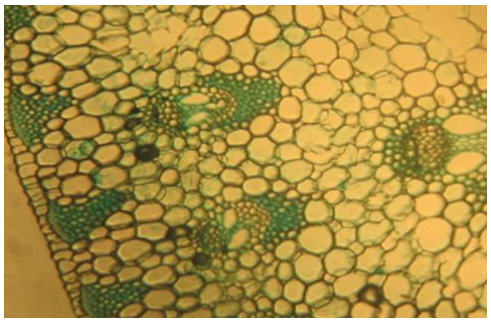
Cyperus rotundus



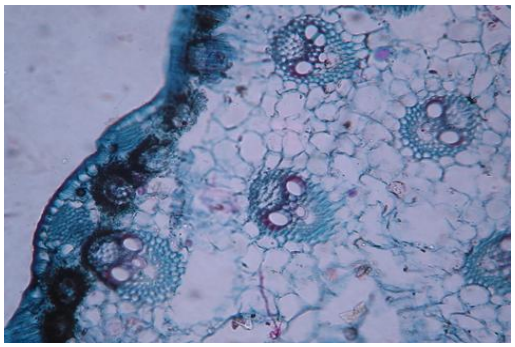
Cyperus squarrosus



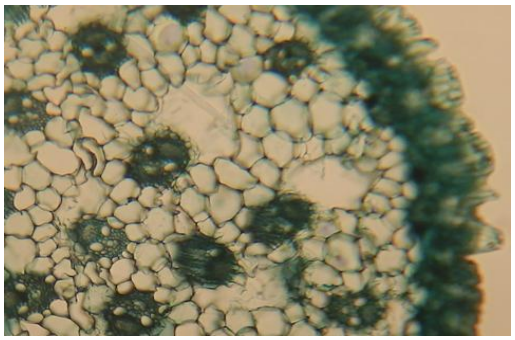
Cyperus iria



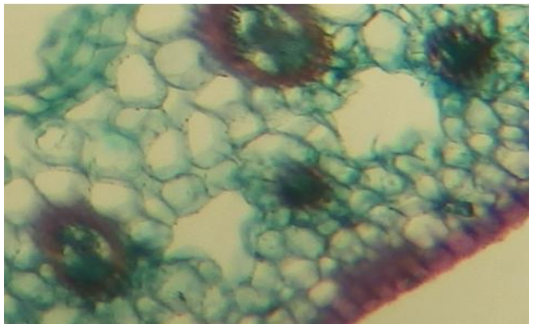
Cyperus alopecuroides



Cyperus compressus



Cyperus laevigatus



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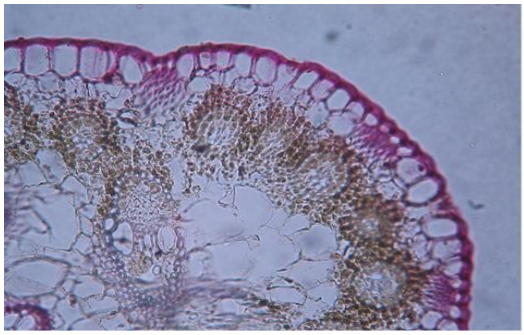
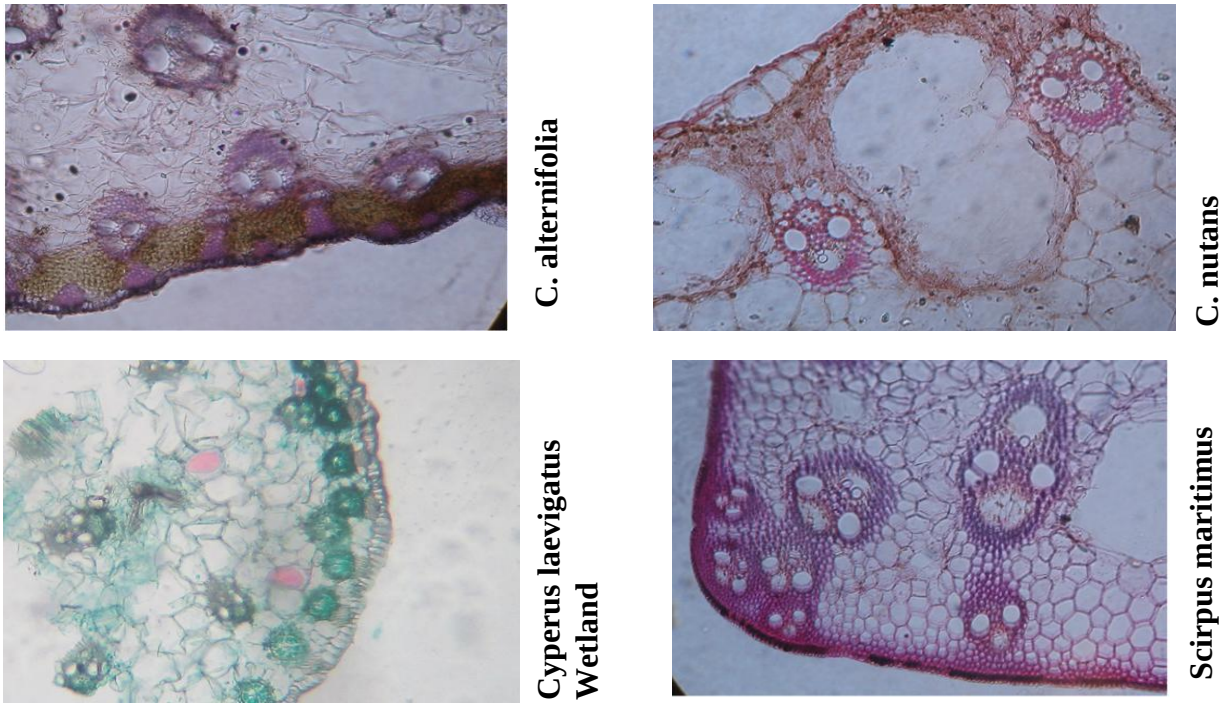


C. esculuntus



Figures: TS of the leaf of some species of family Cyperaceae from Punjab.

Conclusion

The diverse habitat of *Cyperus* species has caused a variety of anatomical modifications in different plants. Adaptations of stem area, vascular bundle thickness, epidermal thickness and cell area, width of aerenchyma, collenchyma and sclerenchyma tissues and thickness of phloem and metaxylem are needed according to the type of soil in which the plant is growing. Plants with better modifications showed accelerated growth even in harsh climate and soil conditions while those with limited changes in anatomy showed moderate or no growth in challenging environments. This study showed different stem anatomies of *Cyperus* species growing in different soils of various regions of Punjab. The spread of different species of genus *Cyperus* of Cyperaceae family in the different regions of Punjab was based on soil type with the relevant modifications in stem anatomy.

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