

## MODIFICATION IN FAMILY CYPERACEAE FOR ABIOTIC STRESS TOLERANCE

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### Abstract

*Cyperaceae* found in various ecotypes of Pakistan as well as worldwide regions. Present study is based on some structural modification in root and stem anatomy of some species of *Cyperaceae* for abiotic stress tolerance. Members of *Cyperaceae* are adapted to a diversity of habitats in Pakistan, such as arid and semi-arid regions (*Cyperus arenarius*, *C. rotundus*), aquatic wetlands (*Scripus* spp. and *Fimbristylis* spp.) and sub-mountainous cooler regions salt range (*Carex* spp.). *Cyperus alternifolia* is the widely cultivated species in Pakistan as ornamental. All the studied species showed some specific structural adaptations to withstand environmental hazards. In the anatomical structure, some species (*Cyperus arenarius*) showed intensive sclerification (1.5  $\mu\text{m}$ ) in the cortical region of root as compared to other species, as well as in the vascular region, tightly-packed cortical parenchyma, maximum Aerenchyma cell area (12-15  $\mu\text{m}^2$ ) in root recorded in *Cyperus alternifolia* and *Cyperus compressus* species. While in the stem anatomy *Carex fedia* showed intensive sclerenchyma (2.5  $\mu\text{m}$ ), cortical thickness and cell area, epidermal thickness almost equal in all species in stem anatomy. These modifications are important not only for minimizing water loss through roots but also for water conservation in harsh environmental condition. Halophytic/salt tolerant species are equipped with intensive aerenchyma for gaseous exchange in waterlogged condition and cortical region with well-developed sclerenchyma that conserve water. In addition, stellate cells are also recorded. These help in water conservation, salt dilution and salt transport.

**Keywords:** Abiotic Stress, Drought, Epidermal Cell, Halophytic, Microstructures, Sclerenchyma, Vascular Bundles

### Introduction:

*Cyperaceae* is the third leading family among monocotyledons and contains about 104 genera with 5,500 species and found in various ecosystems throughout the world (Andrade *et al.* 2023; Bezerra *et al.* 2023; Goetghebeur, 1998; Martins and Scantena, 2013). There are 179 species in Pakistan, divided into 22 genera, the majority of which are weedy species (Kukkonen, 2001). They are widely distributed, with

the tropical areas hosting the majority of their genera. *Carex*, which has 2000 species, is the biggest genus, followed by *Cyperus*, which has 600 species. In the world wide regions it has great economic importance at a regional or local level (Larridon *et al.* 2021; Simpson and Inglis, 2001; Bryson and Carter, 2008).

*Cyperaceae* plants are perennial, seldom annual, rhizome creeping, often stoloniferous, sometimes tuberiferous or bulbous. Mostly have scabrous, firm, triangular, hairless throughout, often

cylindrical, smooth stem. The adventitious root system is characteristic of *Cyperaceae* (Larridon *et al.* 2022; Lima and Menezes, 2008). Mostly have basal or subbasal leaves with sheaths, ligules, or neither, with glabrous blades in most cases, spike, many spikes, or a substantial compound Inflorescence. Bisexual or monosexual flowers are seldom dioecious (Starr *et al.* 2021; García-Moro *et al.* 2022; Mishra *et al.* 2016; Kukkonen, 2001; Ball *et al.* 2002; Dai *et al.* 2003). Sedges may thrive in a range of environments, including wetlands and poor soils as well as contaminated soils, hypersaline waterways, and dry land salinities and it has most important and dominating characteristics Kranz anatomy among the taxonomy and phylogeny group which shows structural and anatomical modification in related to C<sub>4</sub> photosynthesis (Seago *et al.* 2005; Khan and Qaiser, 2006; Martins and Scantena, 2013).

*Cyperus rotundus*, *Cyperus esculentus*, and *Cyperus alternifolia* are only a few of the species that have undergone anatomical research (Taheri *et al.* 2021; Gifford and Bayer 1995; Wills and Briscoe 1970; Wills *et al.* 1980). Vascular bundle containing vessels in the root xylem; simple or scalariform end walls; Vascular bundles have a collateral form and a double-layered bundle sheath with a parenchymatous outer layer and a highly sclerotized, fibrous inner layer (Watson and Dallwitz, 1992; Zhang *et al.* 1998). Uniseriate exodermis and endodermis present with or without thickening, aerenchyma cells with mostly larger gaps continuous or interrupted pericycle are also found (Marques and Moscheta, 2010). The internal cortex formed by radially arranged with thickening of cell and endodermis shows casparian strip as a barrier (Guillaud 1878; Van Tieghem 1898; Starr and Ford, 2001; Bagniewska-Zadworna and Zenkteler, 2006; Lima and Menezes, 2008).

According to several studies (Zhang *et al.*, 2022; Waadt *et al.*, 2022; Gong *et al.*, 2020; Hasanuzzaman *et al.*, 2020; Munns *et al.*, 2006; Saleem *et al.*, 2011), abiotic stressors are a key factor in agricultural production loss. One of the most significant abiotic stressor that negatively impacts plant metabolism and gene expression is salinity and water deprivation (Qari and Tarbiyyah, 2021; Chaudhry and Sidhu, 2022; Anzano *et al.* 2021 and Sidhu 2022). Mahadavi and Sanavy, (2007) beyond these anatomical studies have great importance to cope these abiotic stresses and identified new species which are resistant to abiotic stress by adopted and formation of modified anatomical structures. Present study is based on objectives that how anatomical features of various *Cyperu* species are specialized for stress tolerance in different regions of Pakistan.

#### Materials and Methods

Detailed and random surveys were conducted in diverse areas of Punjab province of Pakistan to collect different species of *Cyperaceae* for the study of root and stem's anatomy (Table 1)

For stem anatomical research, a piece of stem from the center of the stem was chosen and preserved in FAA (formalin acetic alcohol) solution for 48 hours. One centimeter of root was taken from the base of the thickest root for root anatomical investigations. V/V formalin, 10% acetic acid, 50% ethyl alcohol, and 35% distilled water made up the fixative. Later, for long-term preservation, plant material was moved to an acetic alcohol solution (v/v acetic acid 25% and ethyl alcohol 75%). Permanent slides that were made with double-stained (Safranin and Fast green) were using a free-hand sectioning process. Carl-Ziess camera-equipped compound microscope was used to capture photos.

#### Results

### Root Anatomy

Root anatomical characteristics have been discussed in plate 1-3 and Figures 1-2

#### Epidermis Thickness ( $\mu\text{m}$ ) and its Cell Area ( $\mu\text{m}^2$ )

The maximum epidermis thickness was recorded in two species *Carex fedia* and *Cyperus littoralis*. Moderately thick epidermis thickness was recorded in *C. compressus*. The most minimal character thickness was noted in other remaining five species namely *C. alternifolia*, *C. arenarius*, *C. rotundus*, *F. dichotoma* and *S. maritimus*. On the other hand, epidermis cell area was maximum in three species *C. fedia*, *C. alternifolia* and *S. littoralis*. This was followed by two species, *C. rotundus* and *F. dichotoma* that showed minimum epidermis cell area (**Figure 1**).

#### Cortical Thickness ( $\mu\text{m}$ ) and its Cell Area ( $\mu\text{m}^2$ )

Maximum cortical thickness was recorded in *C. alternifolia* while other species showed moderate cortical thickness. On the other hand, maximum cortical area was recorded in *C. rotundus*, which was much enlarged than that recorded in other species (**Figure 1**). *S. maritimus* had the minimum cell area.

#### Endodermis Thickness ( $\mu\text{m}$ ) and its Cell Area ( $\mu\text{m}^2$ )

Endodermis thickness was the least variable character and recorded almost maximum constant thickness in *C. fedia*, *C. alternifolia* and *S. littoralis*,

however remaining five species showed much reduced endodermis thickness. Endodermis cell area was maximum in *C. fedia*, this was much better than the second best record seen in other species *C. arenarius*. Two species *C. compressus* and *S. littoralis* showed very little variation in their endodermis cell area. *C. rotundus* and *S. littoralis* however had the smallest and much reduced endodermis cell area (**Figure 1**).

#### Aerenchyma Cell Area ( $\mu\text{m}^2$ )

The most enormous aerenchyma area ever seen was in *C. compressus* followed by three species namely *C. fedia*, *C. alternifolia* and *C. arenarius* however, *S. maritimus* showed its minimum area, which was much reduced as compared to other species while no aerenchyma cells recorded in *F. dichotoma* (**Figure 2**).

#### Sclerenchyma Thickness ( $\mu\text{m}$ )

A stage micrometer-calibrated ocular micrometer was used to capture the anatomical parameters of the mechanical and circulatory tissues, as well as the root exodermis and endodermis. For the purpose of comparing means, the data were submitted to analysis of variance (Steel *et al.*, 1997).

*Cyperus arenarius* had the maximum sclerenchyma thickness, which was much thicker as compared to other species. The sclerenchyma thickness was greatly reduced in *S. maritimus* and no sclerenchyma cells recorded in *S. littoralis* (**Figure 2**).

**Table 1:** Details of Some Species of *Cyperaceae* Collected From the Punjab, Pakistan

Sr No.	Species	Vernacular Name	Collection Site with Coordinates
1.	<i>Carex fedia</i> (Nees ex Wight)	Valerian Sedge	Jahlar Lake, 32 29'N, 72 07'E, 950m
2.	<i>Cyperus alternifolia</i> (L.)	Umbrella sedge	Old Botanical Garden, University of Agriculture., Faisalabad 31, 25, 43.66N, 73, 04, 16.50E Alt: 187m
3.	<i>Cyperus arenarius</i> (Retz)		Salt range, Uchali lake 32 33'N, , 72 01'E, 700m
4.	<i>Cyperus compressus</i> (L.)	Poorland flat sedge	Head Qadirabad, 32°19' 0" N (32.32°) 73 39' 0" East (73.65°)
5.	<i>Cyperus rotundus</i> (L.)	Motha or Deela, purple nutsedge	Old Botanical Garden, University of Agriculture., Faisalabad 31, 25, 43.66N, 73, 04, 16.50E Alt: 187m
6.	<i>Fimbristylis dichotoma</i> (L.) Vahl	Forked fimbry	Sahianwala, Faisalabad 31, 35, 51.41N, 73, 14, 32.74E Alt: 189m
7.	<i>Scirpus littoralis</i> (Schrud.)	Bulrush	Layyah 30.9693° N, 70.9428° E
8.	<i>Scirpus maritimus</i> (L.)	Salted bulrush	Sahianwala, Faisalabad 31, 35, 51.41N, 73, 14, 32.74E Alt: 189m

### Vascular Bundle, Metaxylem and Phloem area ( $\mu\text{m}^2$ )

*Cyperus alternifolia* showed much enlarged metaxylem vessel area than other species. Its minimum was recorded in *S. littoralis*, which showed very much reduced metaxylem vessel area. Phloem area was considerably greater in *C. rotundus* as compared to other species which almost showed equal phloem vessel area, while *C. alternifolia* had the minimum phloem area. It's followed by total vascular bundle area maximum in two species *F. dichotoma* and *C. compressus* and minimum in *S. littoralis* (Figure 2).

### Stem anatomy

Stem anatomical characteristics have been discussed in plate 4-6 and Figures 3-4

### Epidermis Thickness ( $\mu\text{m}$ ) and its Cell Area ( $\mu\text{m}^2$ )

There were no distinctive variations recorded in epidermis thickness of four species namely *C. fedia*, *C. alternifolia*, *C. arenarius* and *C. rotundus* in which *C. fedia* had the thickest epidermis than other species.

It was followed by *Cyperus rotundus*, *F. dichotoma* and *S. littoralis*, however had moderately thick epidermal layers. Its minimum was seen in *S. maritimus*. Epidermal cell area showed various pattern as was noted in case of epidermal thickness, the amount of variations was greater in case of epidermal cell area. Its maximum was verified in *C. fedia* and *C. compressus* (Figure 3). However, all the other species showed smaller epidermal cells, where the least cells were noted in *S. maritimus*.

### Cortical Thickness ( $\mu\text{m}$ ) and its Cell Area ( $\mu\text{m}^2$ )

There were no distinctive variations recorded in cortical thickness. The maximum cortical thickness was recorded in three species *Carex fedia*, *Cyperus arenarius* and *C. rotundus*, followed by other species that showed moderately thickness and *F. dichotoma* had minimum cortical thickness. In case of cortical cell area *C. alternifolia* showed greater cell area followed by *C. arenarius* had also greater cell area and minimum cell area was recorded in *Carex fedia* (Figure 3).

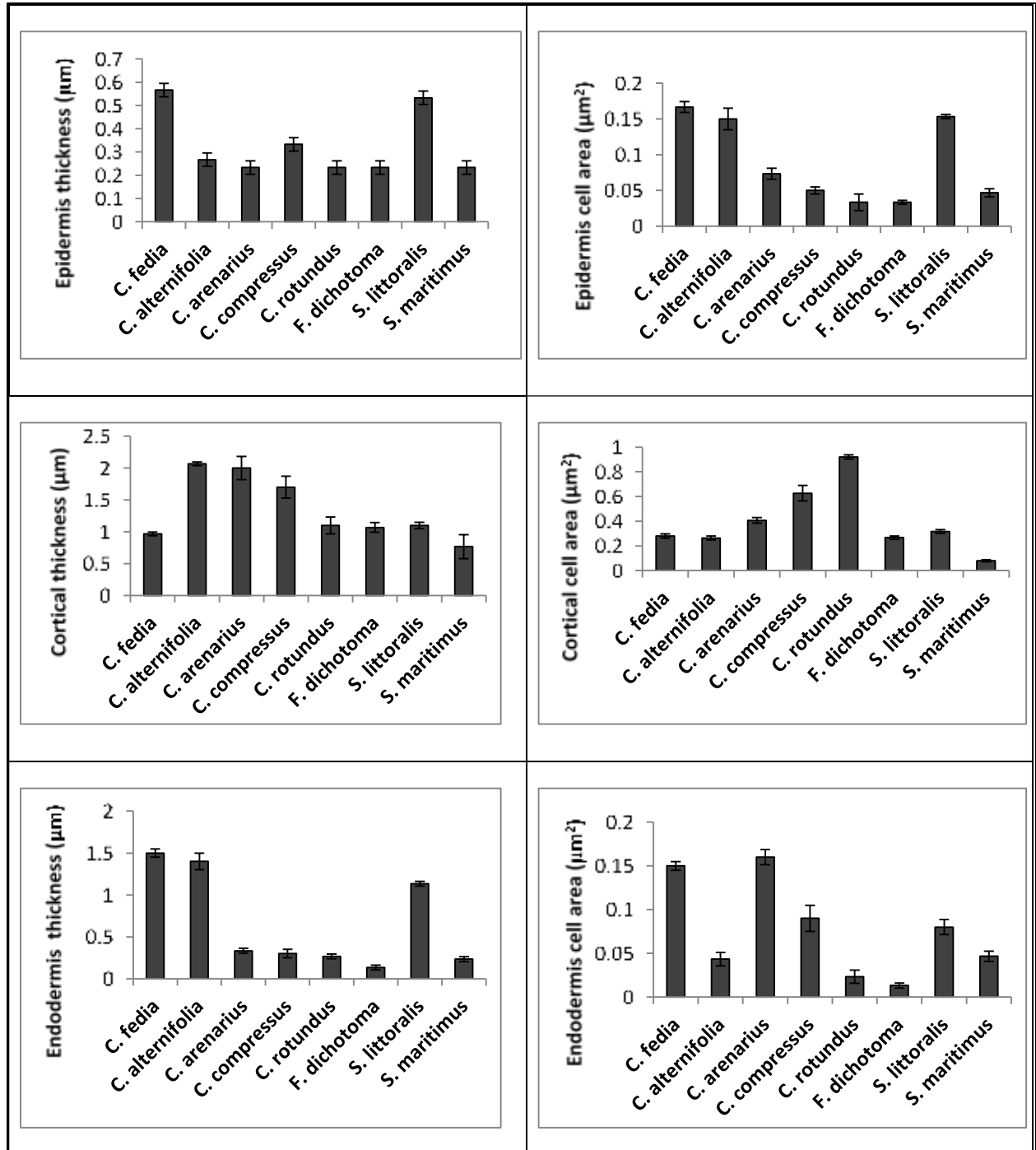


Figure 1: Root anatomical characteristics in different species of *Cyperceae* from various region of Punjab, Pakistan

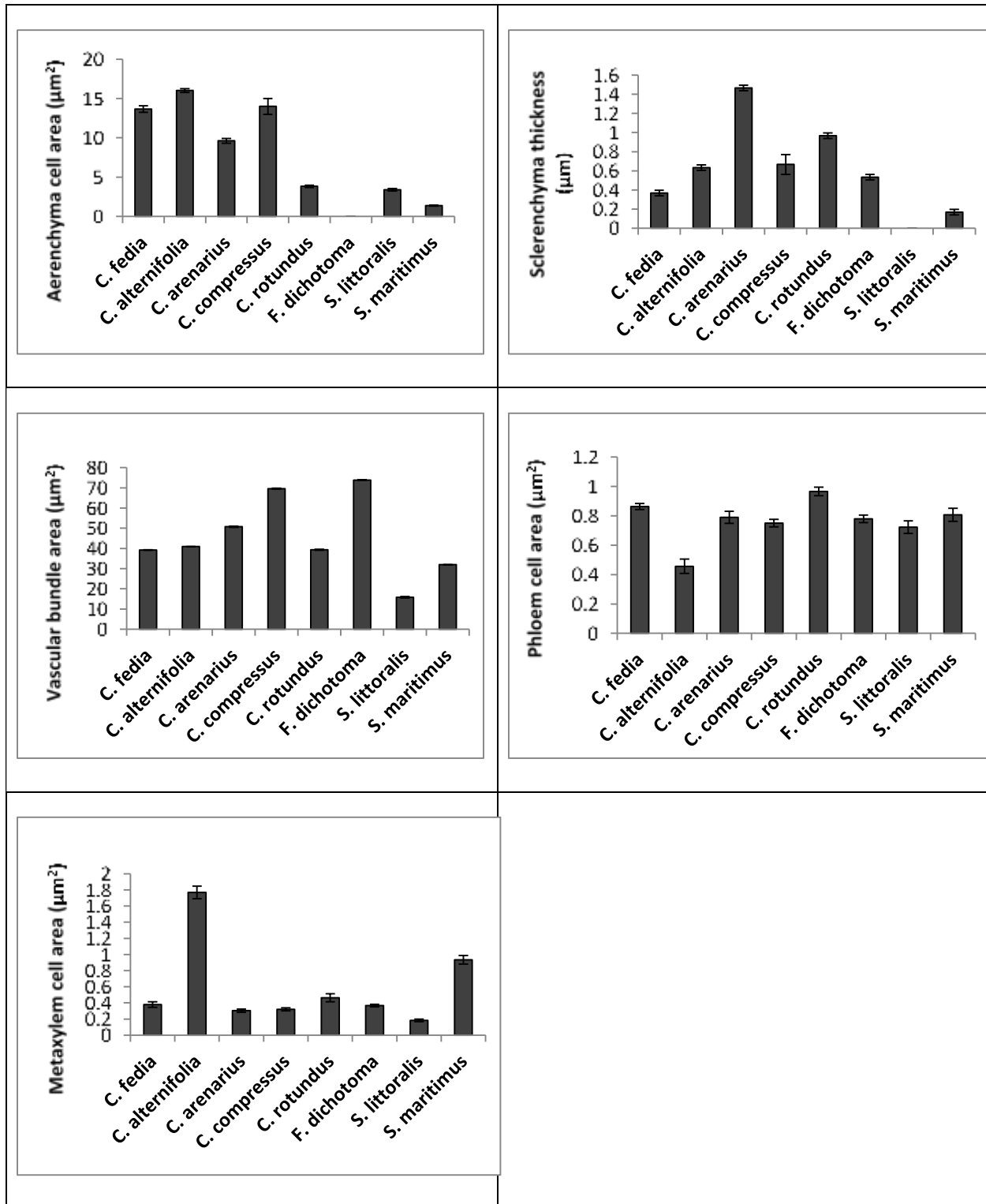


Figure 2: Root anatomical characteristics in different species of *Cyperceae* from various region of Punjab, Pakistan

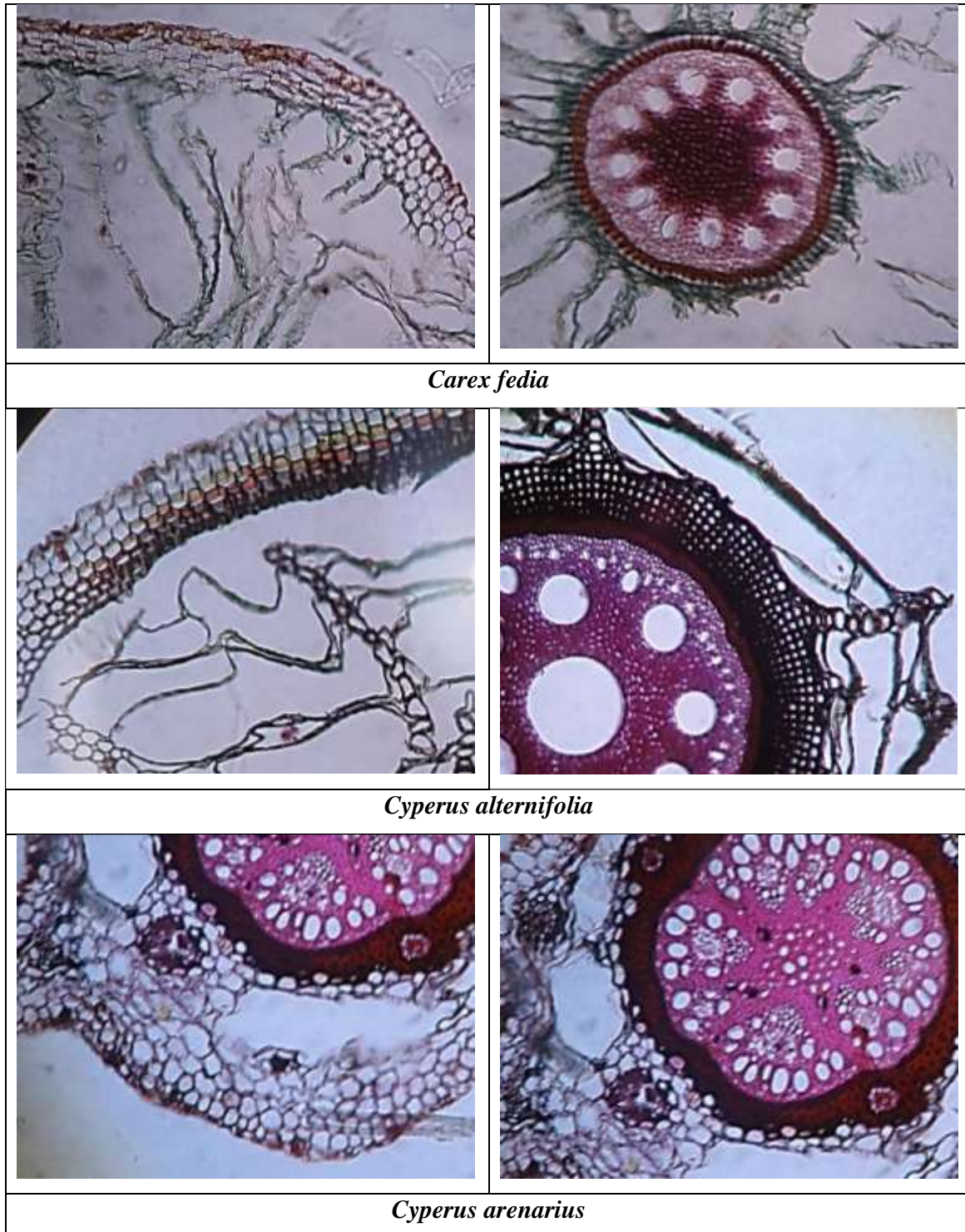


Plate 1. Transverse section of Root in different species of *Cyperaceae* from various region of Punjab, Pakistan

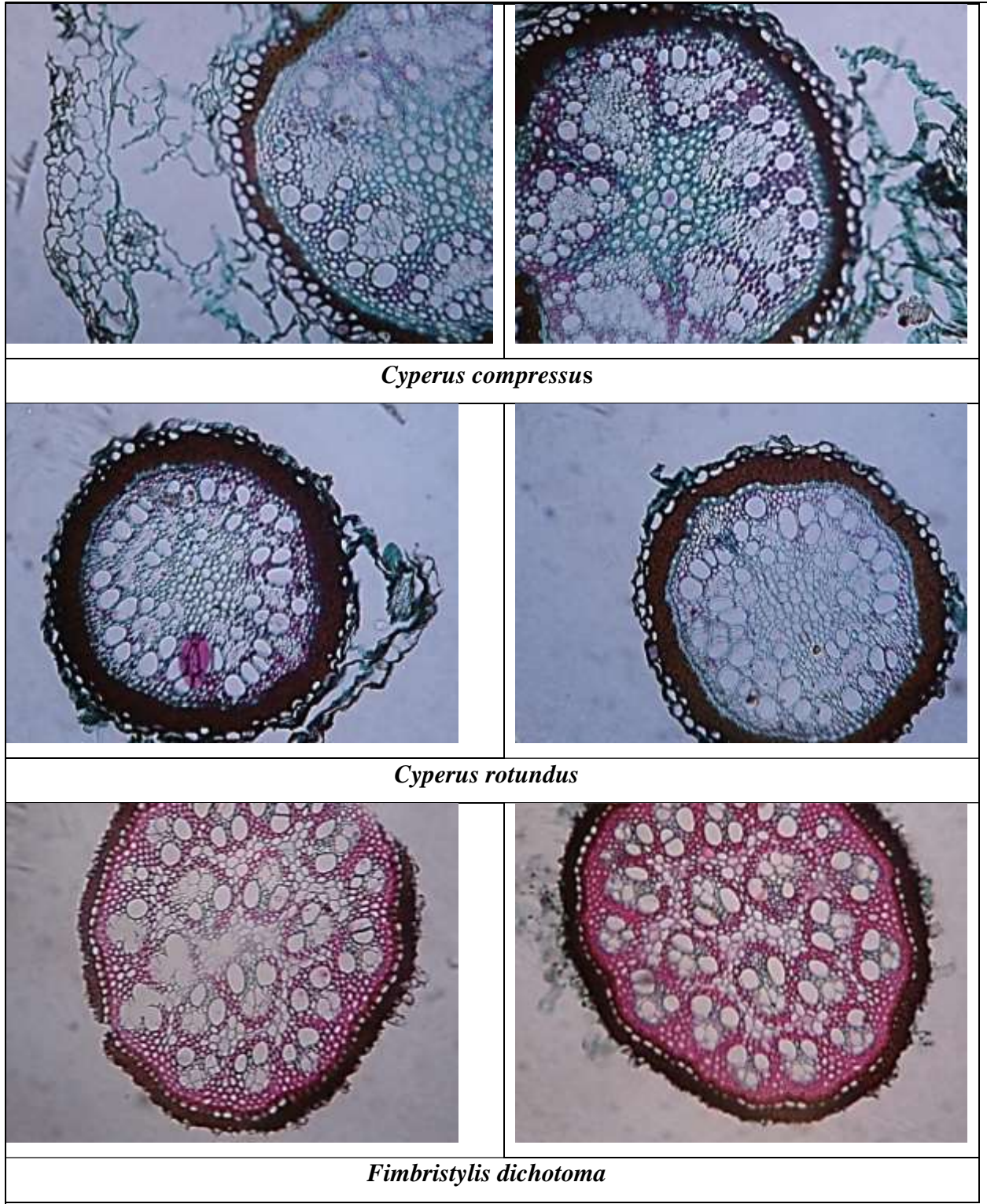
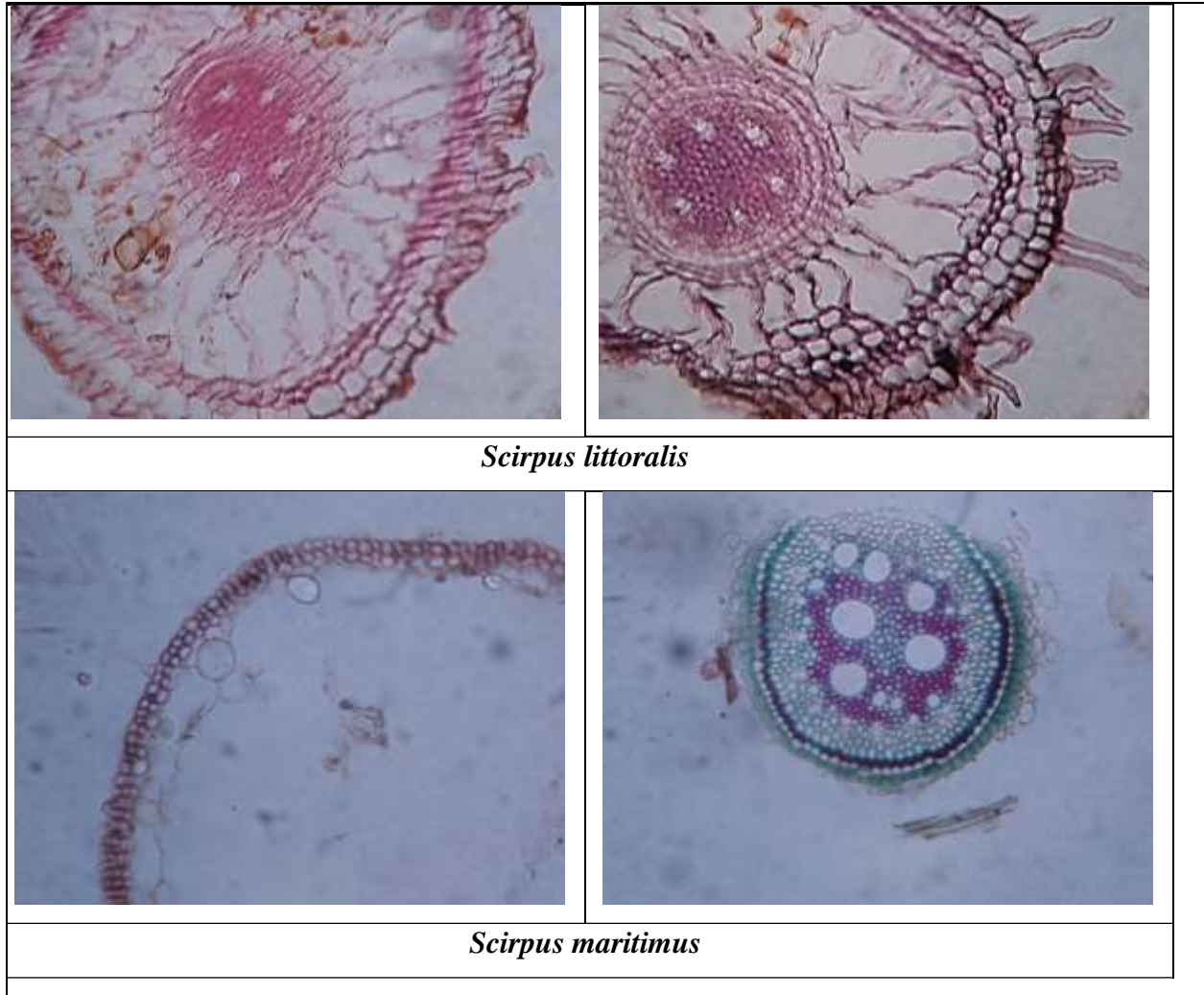


Plate 2. Transverse section of Root in different species of *Cyperaceae* from various region of Punjab, Pakistan





**Plate 3. Transverse section of Root in different species of Cyperceae from various region of Punjab, Pakistan**

**Sclerenchyma Thickness ( $\mu\text{m}$ )**

Sclerenchyma showed greater variation, the maximum sclerenchyma thickness was recorded in *Carex fedia*, however *C. alternifolia* showed moderate thickness while three species namely *C. arenarius*, *S. littoralis* and *S. maritimus* had sclerenchyma of same thickness. The minimum sclerenchyma thickness was recorded in *C. rotundus* (Figure 4).

**Vascular bundle, Metaxylem and Phloem area ( $\mu\text{m}^2$ )**

The maximum area of the vascular bundle of stem was recorded in two species, *C. fedia* and *S*

*maritimus*. The second best vascular bundle area was noted in *S. littoralis*. *C. compressus*, *C. alternifolia* and *F. dichotoma*, all had almost same vascular bundle cell area. *C. arenarius* and *C. rotundus* had the minimum vascular bundle cell area (Figure 4). *Cyperus alternifolia* showed much enlarged metaxylem vessel area than other species. Both *C. arenarius* and *C. rotundus* had highly reduced metaxylem vessels. Phloem area in *Carex fedia* was extremely greater as

compared to other species. It was followed by *F. dichotoma* and *S. maritimus*, while in *C. rotundus* phloem area was much reduced.

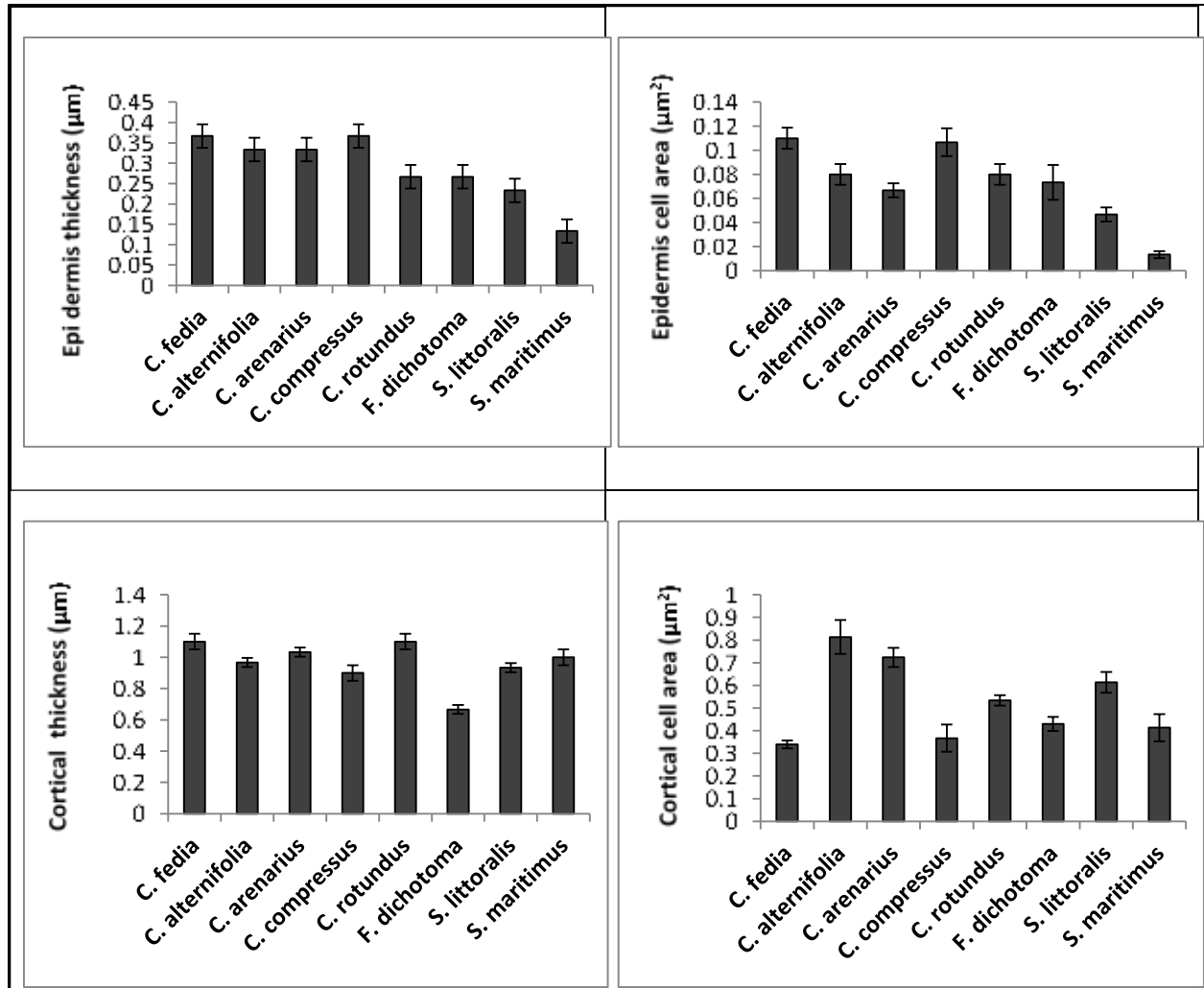


Figure 3: Stem anatomical characteristics in different species of *Cyperceae* from various region of Punjab, Pakistan

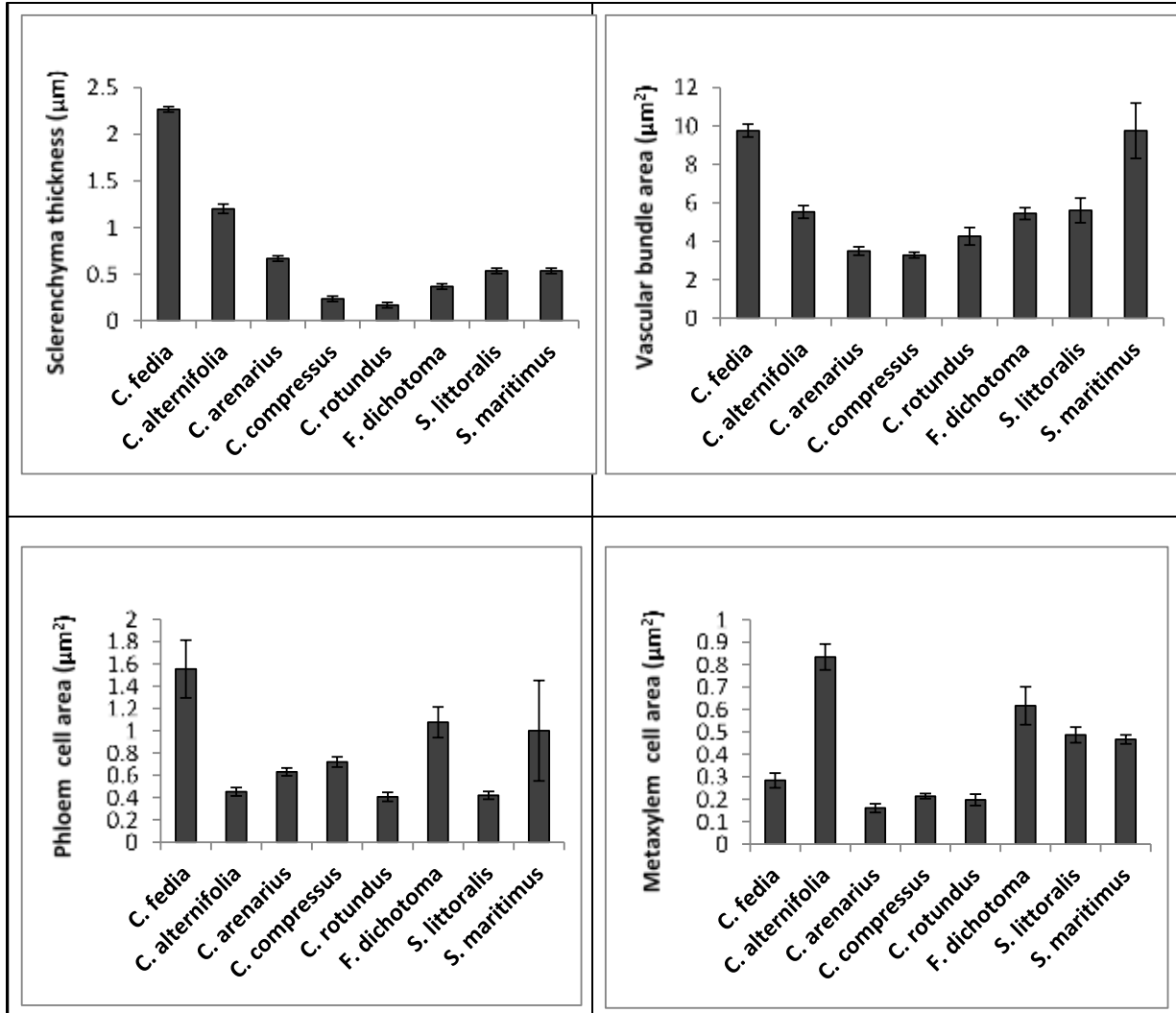


Figure 4: Stem anatomical characteristics in different species of *Cyperaceae* from various region of Punjab, Pakistan

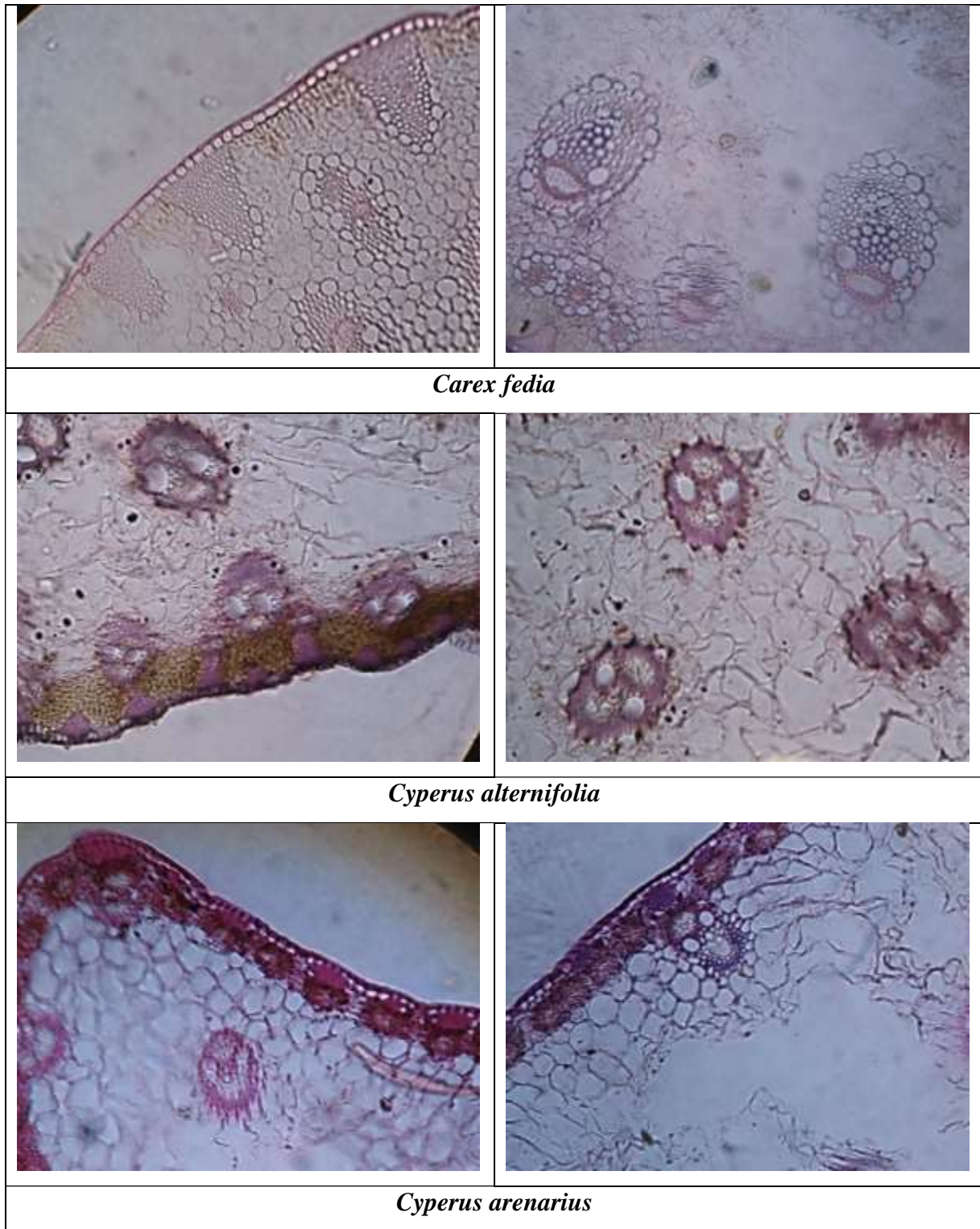


Plate 4. Transverse section of Root in different species of *Cyperaceae* from various region of Punjab, Pakistan

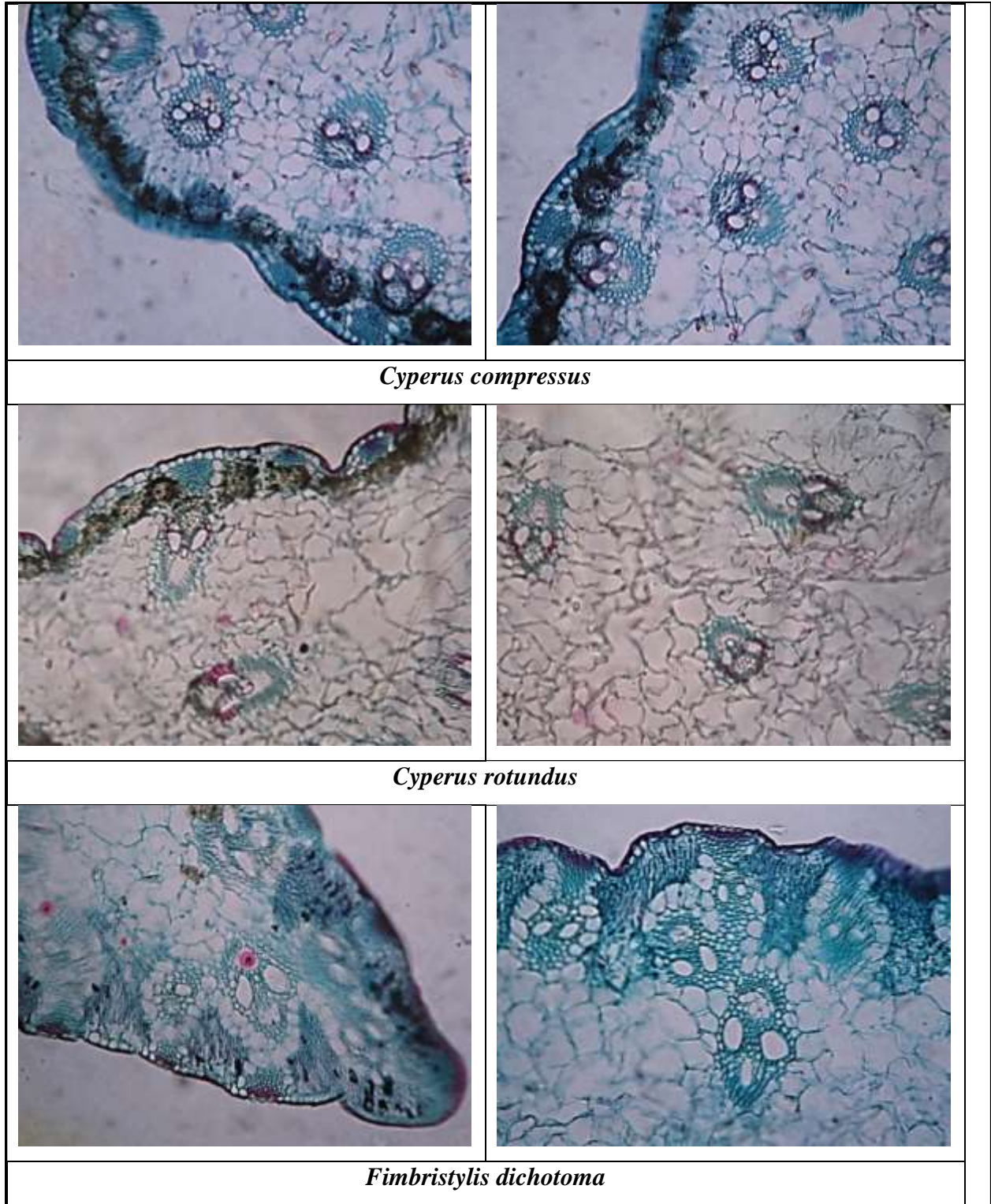


Plate 5. Transverse section of Root in different species of *Cyperaceae* from various region of Punjab, Pakistan

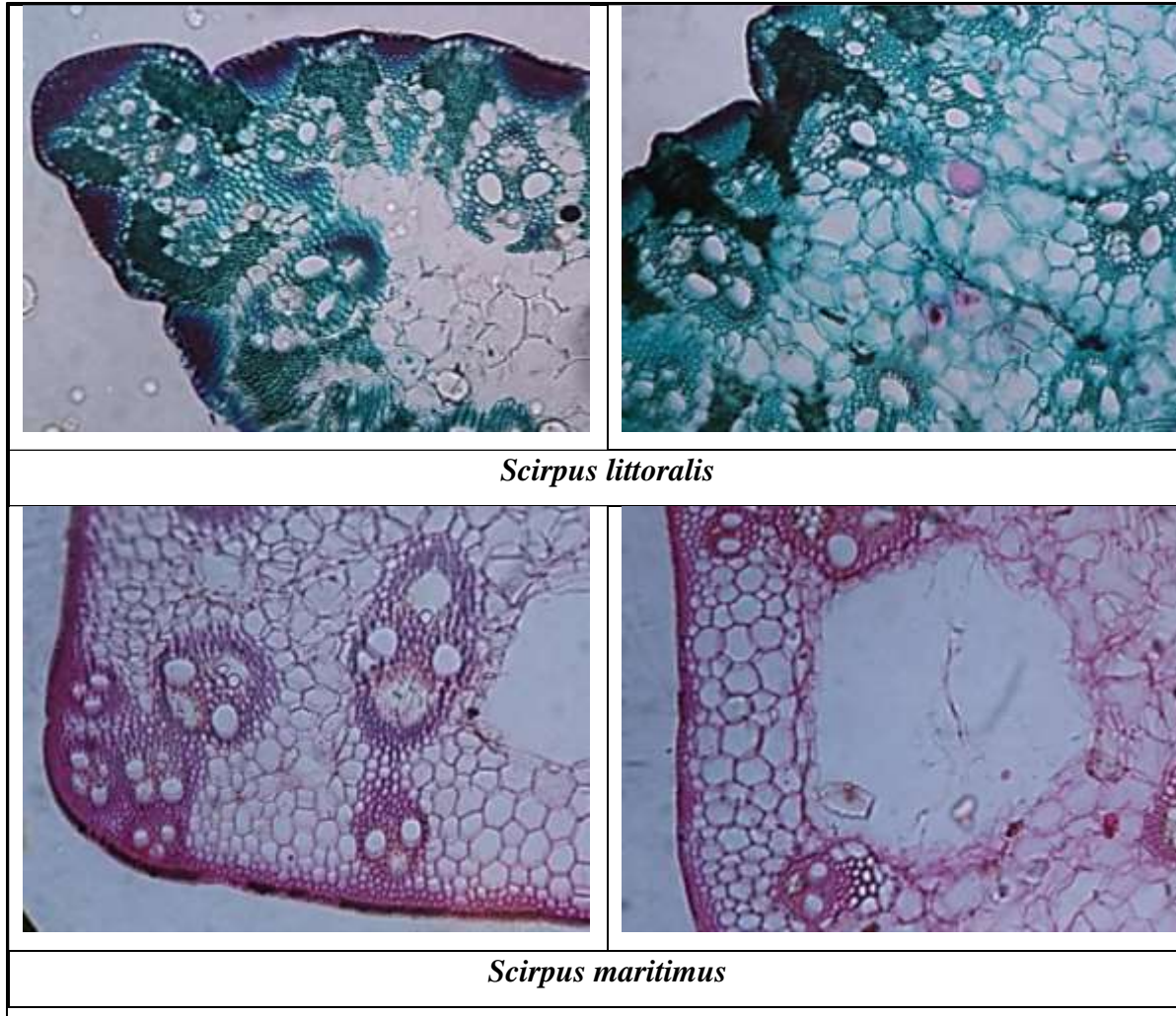


Plate 6. Transverse section of Root in different species of *Cyperceae* from various region of Punjab, Pakistan

## Discussion

*Cyperaceae* family is the most important family after *Poaceae*. *Cyperus* species which have been collected from various habitats showed particular variation in anatomical characters of root and stem which is major reason to adopt various harsh environmental conditions. These adaptations include thick epidermis, large metaxylem vessels, thick sclerenchyma, large aerenchyma cells for gaseous exchange and bulk salt movement. *Carex fedia* and *Cyperus littoralis* has maximum epidermis thickness in roots and stem which is valuable adaptation against salt stress and water scarcity to conserve water.

Same results discussed by Botti *et al.* (1998) and YuJing *et al.*, (2000) that Halophytic plants often develop thick epidermis, which acts as a powerful defense against decreased water supply. Thick epidermis is a trait of salt-tolerant species that shown its greater adaptation in the Salt Range population (Awasthi and Pathak, 1999). Epidermal cell area was maximum in three species *C. fedia*, *C. alternifolia* and *S. littoralis* in roots and in stem of *C. fedia* and *C. compressus*. The epidermal cell area of Salt Range population was expanded to stop water loss via the stems (Hameed *et al.*, 2010). In contrast to Akram *et al.* (2002), salinity normally reduced epidermal cell area in stem.

Kulkarni *et al.* (2008) also studied that *Fimbristylis dichotoma* aquatic halophytic species broadly which dispersed in countries with tropical and subtropical climates, as well as in locations with a high salt content (Siwakoti and Tiwari, 2007). Thicker epidermises in a number of *Eucalyptus* species are better capability to survive in harsh climatic conditions, the

adaptations like drought and salinity (Ali *et al.* 2009; Jenks and Ashworth, 1999; Ristic and Jenks, 2002).

In root cells cortical thickness was maximum in *Cyperus alternifolia* which was also recorded from marine habitats (Lillebo *et al.*, 2003) and it is only one cultivar of it has been identified in the Punjab area (Hameed *et al.*, 2010) while in stem cells cortical thickness was higher in *Carex fedia*, *Cyperus arenarius* and *C. rotundus*. In salt range salt tolerant plant's cortical cell increased (Hameed *et al.*, 2010). According to (Akram *et al.*, 2002) who observed cortical cell area decreased at high salinity level but increased in salt range population to enhance storage capacity (Baloch *et al.*, 1998). *E. microtheca*, *F. Muell* and *E. botryoides* have huge cortical cells as an evidence of their large allocation in various environmental circumstances (Ali *et al.*, 2009).

Sclerenchyma thickness which is most important anatomical adaptation against abiotic stress and *Carex fedia* showed maximum thickness in stem cells while in root cells *Cyperus arenarius* showed better adaptability against harsh environment. Salt tolerant species has thick endodermis, parenchyma and lignified sclerenchyma cells (Baloch *et al.*, 1998; YuJing and Yong, 2000; YuJing *et al.*, 2000; Hameed *et al.*, 2010). *Cyperus compressus* followed by three species namely *C. fedia*, *C. alternifolia* and *C. arenarius* showed maximum aerenchyma cell area which is another valuable anatomical and physiological adaptation against abiotic stress; work as gaseous exchange and for bulk salt movement.

It was also discussed by Muhlenbock *et al.* (2007) that in *Cyperus alternifolius* cultivated

species aerenchyma cell enhance gaseous exchange and water storage capacity.

Root and stem cells of *Cyperus alternifolia* showed higher metaxylem area and *Carex fedia* has large phloem area for water and nutrient movement. Larger protoxylem vessels may enhance the conduction of water and nutrients (Hose *et al.*, 2001; Cholewa and Griffith, 2004). This has been supported by Hameed *et al.* (2010) who found that salt range species are most salt tolerant and has large metaxylem and when the environment is hostile, the phloem region may participate in the conduction of water and photosynthates.

This has also been reported in various other plant species e.g. in rice (Datta and Som, 1973; Zhang *et al.*, 2013), *Kandelia candel*, *vaccinium corymbosum* (Hwang and Chen, 1995; Luis *et al.*, 2008), Bougainvillea (Chew, 2010), *Arabidopsis thaliana* (Baloch *et al.*, 1998), *Ziziphus* cultivars (Awasthi and Pathak, 1999), *Urginea grandiflora* Bak and *Pancratium tortuosum* Herbert (Sultan *et al.*, 2010) and *Eucalyptus* species (Ali *et al.*, 2009). Sometimes embolism occurred due to larger metaxylem vessel, in this condition smaller vessels prevent from it under drought condition (Facette *et al.*, 2001).

### Conclusion

*Cyperaceae* has number of anatomical modification for its better adaptability against abiotic stressed harsh environmental conditions like increased cortex and pith parenchyma for greater water storage, maximal epidermis and sclerenchyma thickness for reducing water loss via root surface, and endodermis for inhibiting radial passage of water and nutrients. It had

increased the stem's succulence, the thickness of the epidermis and sclerenchyma to prevent water loss, the thickness of the cortex to improve water storage, aerenchyma for exchange of gasses, the number of vascular bundles, and the area of the metaxylem, protoxylem, phloem, and sieve to improve the flow of water, nutrients, and photosynthate. These features are used as the anatomical markers to evaluate the stress tolerance in various environmental conditions.

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