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Anatomical adaptations in species of Poaceae growing in Al-Ha'ir region of Riyadh, Saudi Arabia

Shamah A. Banan, Ahlam A. Al-Watban, Abdullah R. Doaigey, Abdulaziz A. Alsahli* and Mohamed El-Zaidy

Department of Botany and Microbiology, College of Science, King Saud University P. O. Box 2455, Riyadh 11451, Saudi Arabia.

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The aim of this work was to determine the anatomical adaptations of leaves and stems of four species of *Poaceae* (*Cynodon dactylon* L. Pers., *Chloris barbata* SW. (Chloridoideae), *Setaria verticillata* L. P. Beauv., and *Panicum coloratum* L. (Panicoideae)) growing naturally at Al-Ha'ir region near Riyadh, Saudi Arabia. Cross-sections of the leaves revealed that the epidermis cells were spherical to oval and formed one layer with a thick cuticle as well as numerous bulliform cells in a fan shape and prickles. Ground tissue (mesophyll) consists mostly of chlorenchyma cells. A large vascular bundle surrounded by two bundle sheaths, outer sheath parenchyma and inner sheath sclerenchyma were observed in *C. barbata* and *C. dactylon,* while one bundle sheath of sclerenchyma surrounds the vascular bundle in *S. verticillata* and *P. coloratum*, while one bundle sheath with chlorenchymatous cells surrounded the small vascular bundles. Our results showed that all species contained a Kranz anatomy indicative of a C4 photosynthetic pathway despite belonging to two different subfamilies, *Panicoideae* and *Chloridoideae*. Oil droplets appeared in the mesophyll tissue of *P. coloratum* and *S. verticillata*. Cross-sections of stems revealed an epidermis which consists of one layer of cells with spherical to oval shape and had a thick cuticle. Ground tissue contains strands of chlorenchyma cells followed by sclerenchyma tissue surrounding vascular bundles, thereby making a continuous cylinder. The vascular bundles were scattered in the ground tissue, with each vascular bundle surrounded by a single sclerenchymatous bundle sheath. Our results indicate that these plants were characterized by anatomical adaptations that enhance drought-tolerance capabilities, facilitating survival in arid and semi-arid regions such as Al-Ha'ir and thus these plants can be used to increase vegetation cover and pasture area in dry environments.

Key words: Anatomy, adaptations, Poaceae, *Cynodon dactylon*, *Chloris barbata*, *Setaria verticillata*, *Panicum coloratum*.

INTRODUCTION

The *Poaceae* family comprises a large number of economically important crops such as Triticum and Hordeum (Al-War'a et al., 1997), and several saline-tolerant species, including *Cynodon dactylon* (Poljakoff-

*Corresponding author. E-mail: aalshenaifi@ksu.edu.sa.

Mayber, 1988) In addition, several plants in the *Poaceae* have important medicinal and aromatic benefits; for example, dried *C. dactylon* leaves are often used in anabolic and antiseptic substances (Gangwar et al., 2011), and the cereals *S. italica* (L.) P. Beauv is used to treat chicken pox in some areas of Pakistan (Ahmad et al., 2011). Moreover, *C. barbata* has been shown to have antibacterial activity (Natrajan et al., 2012). Several terrestrial plant species of family Poaceae were studied from the anatomical point of view (Kellogg, 2015; Panizzo et al., 2017). The plant species in our study were *C. dactylon* L. Pers. and *Chloris barbata* SW that belong to the *Chloridoideae* subfamily, while *P. coloratum* L and *S. verticillata* P. Beauv were part of the *Panicoideae* subfamily of the *Poaceae* family. These plants were considered permanent herbs, with the exception of *S. verticillata*, grow wild in Al-Ha'ir, an area south of Riyadh city in Saudi Arabia. However, the morphological characteristics of these species can vary significantly. Peng et al. (2017) studied the anatomical feature of swamp plant species that reflects the adaptation of plants to different environmental variables paralleled with morphological, eco-physiological and growth responses. Many studies have focused on the anatomical aspects of the *Poaceae*, such as Shaheen et al. (2012), who observed differences in leaf epidermal characteristics among six *Panicum* spp. native to Pakistan; these included variations in the shape of the phytoliths, prickles, large and small trichomes, and stomata, factors that were often used as important taxonomic indicators at the genus level. Ahmad et al. (2011) in a study of leaf epidermal characteristics of five plant species in Pakistan, including *C. barbata* and *C. dactylon*, found that differences in stomatal apparatus, subsidiary cells shape, the long epidermal cells, and silica cells were useful for distinguishing among species. Hameed et al. (2010) examined the anatomical adaptations of *C. dactylon* collected from saline and non-saline environments in Pakistan, reported that adaptations in plants growing in saline environments included saline excretions on the plant surface, accumulation of ions in the parenchyma tissue, reductions in stomata size on the leaf upper epidermis, increased bundle sheathing, and increased leaf bulliform cells size. Leaves epidermal characteristics of 13 species within 10 genera of Poaceae were studied by Nazir et al. (2013). They characterized the plants that grow in coastal zones by the presence of rows of silica bodies, which differed in shape and size among the plant species, silica bodies were cross- or dumb-bell-shaped or intermediate between the two and accompanied by cork cells.

Vanesa and Cambi (2010) distinguished the occurrence of parenchyma tissue in the midrib region and prickles in leaves of *Trichloris crinita*, and further noted a set of sub-epidermal fibers alternating with chlorenchyma tissue, whereas in *Pappophorum phillippianum* and *T. crinita*, sclerenchyma cells were ring-shaped around vascular bundles. Eltahir and Abuereish (2010) reported the occurrence of small oil droplets in epidermal cells of leaf and mesophyll of *Cymbopogon schoenanthus* and *Cymbopogon citratus*. Carvalho Santos et al. (2013) evaluated variations in anatomical characteristics of 38 different genotypes of *Brachiaria ruziziensis* belong to *Poaceae* to identify genotypes that could be useful in plant breeding. Photosynthesis in C_4 plants was more active under environments that stimulate carbon loss through photorespiration, for example high light intensity and temperatures and reduced water obtainability in the case of stomatal closing (Carmo-Silva et al., 2009). The results of previous studies have shown that plants and grasses of the *Poaceae* were globally ubiquitous and often have great economic, medical and pastoral importance. Research has revealed numerous anatomical modifications among plants, which has greatly improved our understanding of how plants were able to overcome unsuitable environmental conditions and increase their growth and productivity. Our objective here was to determine the anatomical modifications of several of the plant species growing in Al-Ha'ir that contribute to their tolerance to the arid conditions of this part of Saudi Arabia. Improving our understanding of these adaptations may assist in enhancing vegetation cover in desert areas.

MATERIALS AND METHODS

The four plant species we focused on were members of the Poaceae, which grow naturally in Saudi Arabiathese species, consists of *C. dactylon* (L). Pers., *C. barbata* SW. (Chloridoideae), *S. verticillata* (L). P. Beauv. and *P. coloratum* L. (Panicoideae). Plant samples were collected at the flowering stage in the Al-Ha'ir region near Riyadh in the spring of 2017 and identified by botanists in the Herbarium, Department of Botany and Microbiology, King Saud University (KSUH). All samples were kept in 70% ethyl alcohol. Permanent sections were prepared in paraffin wax following the procedures described by Al-Khazraji and Aziz (1989) and Doaigey et al. (1997) for studying the internal anatomical characteristics of leaves and stems of the samples. Sections were examined and photographed with a TK-C1381EG video camera Japan, attached to an Olympus BX4LTF light microscope, Japan.

RESULTS

Leaf anatomical characteristics

Cross-section of the blades of *C. dactylon*, *C. berbata*, *S. verticillate* and *P. coloratum* leaves showed that the leaves were V-shaped, and the vascular bundles appeared in a single row parallel to the lower and upper surfaces of the leaf.

Coastal regions

Upper epidermis

In leaves of all studied species, the upper epidermis

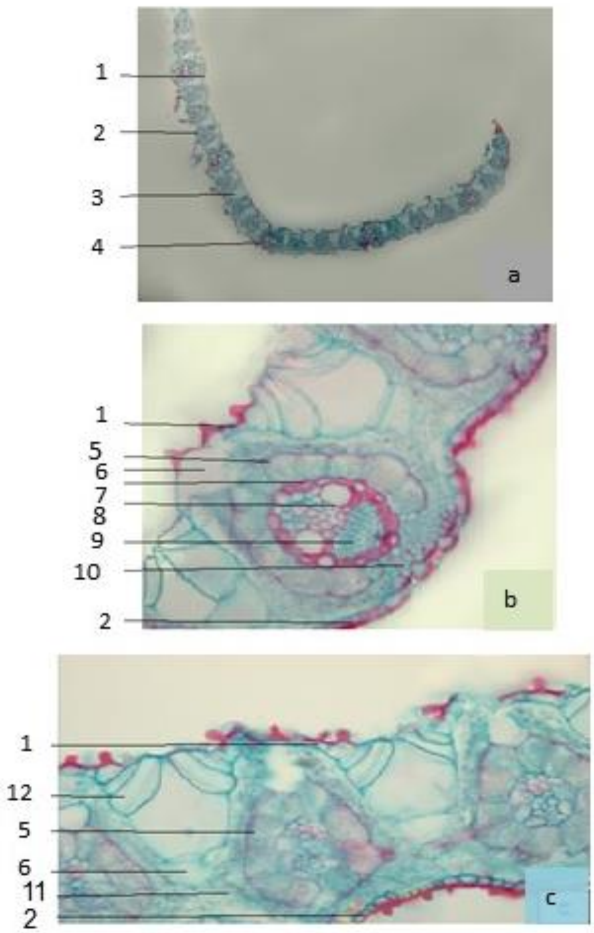

Figure 1. Cross sections of the *C. dactylon* (L.) Pers. (leaf). a-Blade shape 50×, b-Coastal region 400×, c-Intercostal region 400×, 1-Upper epidermis, 2-Lower epidermis, 3-Intercoastal region, 4-Coastal region, 5-Parenchyma shealth, 6-Aerenchyma, 7-Sclerenchyma shealth, 8-Xylem, 9-Phloem, 10-Sclerenchyma cells, 11-Chlorenchyma tissue and 12-Bulliform cells.

consists of one layer of cells spherical to oval shaped, with thick outer walls, thin internal walls and protected with a thick cuticle. The epidermis cells in cross sections of *C. dactylon* appeared to be papillary shaped (Figure 1b), and leaves of *C*. *barbata* contain many prickles (Figures 3a and 3b). In *S. verticillata* leaves, a thick cuticle covered the epidermis that contains bulliform cells (Figure 5a), whereas epidermis cells in leaves of *P. coloratum* were spherical to palisade-shaped, contained numerous large bulliform cells, and covered by a thick cuticle (Figures 7b and 7c).

Ground tissue

In *C*. *dactylon* ground tissue consists of an aerenchyma followed by 2 to 3 rows of chlorenchyma cells, filled with chloroplasts and surrounded by thin cellulose walls (Figure 1b). The ground tissue in *C*. *barbata*, consists of 4 to 7 irregular- and large-sized parenchyma cells with thin cellulose walls free of chloroplasts, followed by 2 to 3 layers of chlorenchyma cells Containing chloroplasts (Figure 3a). Ground tissue in *S. verticillata* consists of 2 to 10 layers of irregularly-shaped parenchyma cells with thin cellulose walls free of chloroplasts followed by 2 to 3 layers of chlorenchyma cells filled with chloroplasts (Figure 5a). Finally, in *P. coloratum*, the ground tissue consists of 4 to 6 layers of irregularly shaped parenchyma cells, free of chloroplasts, with thin cellulose walls followed by 2 to 3 layers of chlorenchyma cells filled with chloroplasts (Figures 7a and 7b).

Vascular tissue

Vascular tissue in the leaves of these species consists of oval-shaped, closed collateral bundles in oval shape. The bundle consists of xylem that is oriented towards the upper epidermis in an approximately V-shape and contains large cavities and thick lignin walls in vessels, with an inverted metaxylem occupying the V-shape head. The phloem, containing companion cells and sieve tubes, was located near the lower epidermis. Large vascular bundles were surrounded by two layers of cells that define the perimeters of the bundle sheaths; its outer layer contained large parenchymal cells, containing some chloroplasts, and surrounded by cellulose thin walls. However, the inner layer was composed of small lignified thick-walled sclerenchyma cells. In the leaves of all the four plants a bundle sheath of parenchyma cells containing chloroplasts were surrounded the small vascular bundles (Figures 1b, 3b, 5a and 7b). Large vascular bundles were connected to the lower epidermis via a group of sclerenchyma cells with lignified walls; sclerenchyma cells in 3 to 5 layers were presented in *C. dactylon* and *P. coloratum* (Figure 7a), 2 to 4 layers occur in *S. verticillata* (Figure 3b) and 5 to 7 layers were found in *C. barbata* (Figure 5a).

Lower epidermis

It was similar in appearance to the upper epidermis in leaves of all four plants, except that prickles were absent in the leaf epidermis in C. *barbata* (Figure 3b).

Intercostal region

Ground tissue

Ground tissues in all plants had 3 to 4 layers of chlorenchyma cells, filled with chloroplasts, and surrounded by thin cellulose walls. The results showed that C. *dactylon* (Figure 1c) was different compared to *C.*

barbata (Figure 3c) in the presence of aerenchyma, whereas *S. verticillata* (Figure 5d) and *P. coloratum* (Figure 7d) were dissimilar in the occurrence of oil droplets in some cells.

Lower epidermis

It was similar to the upper epidermis of the four plants under study in its Characteristics, except the absence of bulliform cells and a thick cuticle in *C. dactylon* (Figure 1c), *C. barbata* (Figure 3c), and *S. verticillata* (Figure 5c). The lower epidermis in ground tissue of *P. coloratum* consisted of a single layer of spherical-to palisade-shaped cells, with thick outer walls covered by a thin cuticle and thin internal walls (Figure 7c).

Stem anatomical features

Cross-sectioned stems were circular in both *C. dactylon* (Figure 2a) and *C. barbata* (Figure 4a), but heart-shaped in *S. verticillata* (Figure 6a) and *P. coloratum* (Figure 8a).

Epidermis

Stem epidermis was composed of a single layer of spherically- to elliptically-shaped cells with thick outer and thin internal walls. Outer walls were covered by a thick cuticle in *C. dactylon* (Figure 2a) and *C. barbata* (Figures 4a and b), but the cuticle was thin in *S. verticillata* (Figure 6a) and *P. coloratum* (Figure 8a).

Ground tissue

For *C. dactylon*, the results showed that ground tissue (chlorenchyma tissue) consists of 2 to 3 layers of thin-walled parenchyma cells containing chloroplasts followed by 3 to 5 layers of sclerenchyma cells in a cylinder shape, which was connected to the external vascular bundle sheath. The remaining ground tissue consists of parenchyma cells free of chloroplasts and with thin cellulose walls, with cell size increasing towards the center of the pith and containing large intercellular spaces (Figures 2a and 2b). Ground tissue in *C. barbata* was composed of 3 to 4 layers of chlorenchyma cells with thin cellulose walls, and containing chloroplasts. Clusters of 3 to 4 layers of sclerenchyma cells with lignin walls were connected to the epidermis, and were followed by 3 to 4 layers of sclerenchyma cells that form a cylinder shape. The sclerenchyma cylinder connects to the sheath of the vascular bundle. The remaining ground tissue consists of thin-walled parenchyma cells free of chloroplasts, with cell size increasing towards the center of the pith, and with clear intercellular spaces (Figures 4a and 4b). In *S. verticillata*, ground tissue consisted of 4 to 5 layers of small parenchyma cells with thin cellulosic walls, which represent the initial ground-tissue layers, along with 3 to 4 layers of sclerenchyma cells with lignified walls, which were connected to the vascular bundles. The ground tissue towards the pith center (Figure 6a and 6b) was similar to that in *C. barbata*; however, crystalline sand was also found in the parenchyma cells (Figure 6c). Finally, in *P. coloratum*, the ground tissue consisted of 4 to 5 layers of chlorenchyma cells with thin cellulose walls containing chloroplasts, along with 4 to 5 layers of sclerenchyma cells that form a complete cylinder, which connect to the sheaths around the vascular bundles. The remaining ground tissue in this species consists of parenchyma cells free of chloroplasts with thin cellulose walls, with cell size increasing towards the center of the pith and containing small intercellular spaces (Figure 8b).

Vascular tissue

Vascular bundles were scattered throughout the ground tissue in all four species, and the stem center was free of vascular bundles, except in *P. coloratum*. The vascular bundles form closed collateral and were oval shaped. Vascular bundles consist of xylem and phloem, with the primary xylem including metaxylem vessels, the latter was located at the head of the V-shape, and vessels with thick lignin walls and large cavities, and protoxylem vessels were replaced by air spaces or protoxylem cavities. The phloem was located toward the outer side and contained companion cells and sieve tubes. The vascular bundles were distributed throughout the primary tissue in all four plant species and were surrounded by sclerenchyma cells in one layer (that is, a bundle sheath; Figures 2a and b; Figures 4a and b; Figures 6a and b and Figures 8a and b, respectively).

DISCUSSION

Our examination revealed several of the internal anatomical adaptations of the leaves and stems associated with higher drought tolerance in *C. dactylon* (L). Pers., *C. barbata* SW, *S. verticillata* (L). P. Beauv. and *P. coloratum* L (Figures 1 to 8). As shown in Figures 1, 3, 5, and 7, the leaf blade was narrow and V-shaped in *C. dactylon* and *C. barbata* (Figure 1a and Figure 3a), but V-shaped and divergent in *S. verticillata* and*, P. coloratum* (Figure 5a and Figure 7a). Our results accord with those of several previous studies in which it was suggested that the U-shaped (Metcalfe, 1960) or V-shaped (Doğan and Tosunoğlu, 1992; Mavİ et al., 2011) leaf blades of members of the *Poaceae* were an adaption to reduce the leaf-surface exposure to light, thus reducing water loss; moreover, the flat blade may facilitate absorption of vital minerals at times when water was plentiful.

Our analyses of leaf cross-sections (Figures 1, 3, 5,

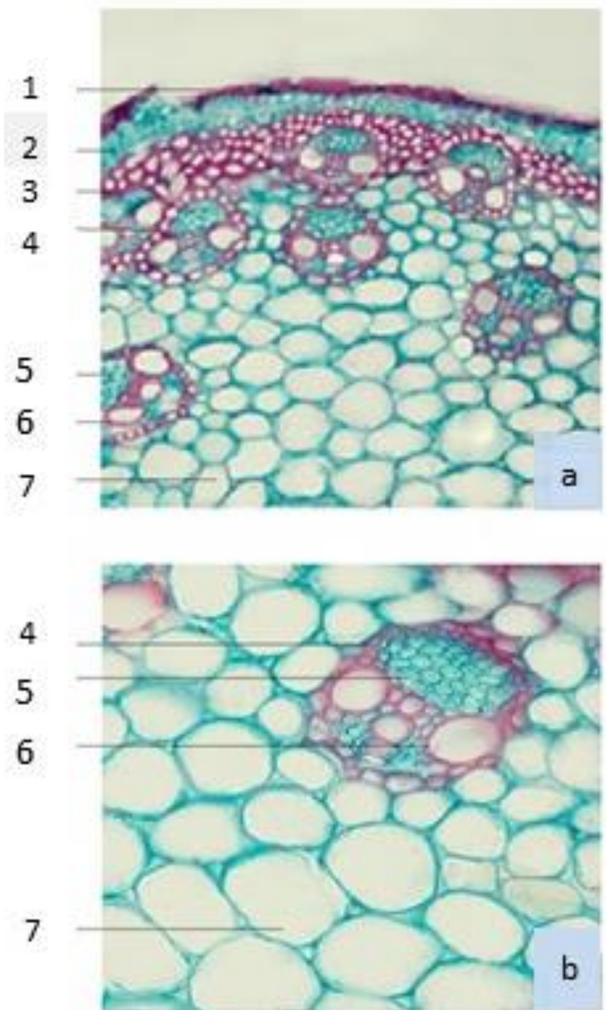

Figure 2. Cross sections of *C. dactylon* (L.) Pers. (Stem). a-200×, b-400×. 1-Epidermis, 2-Chlorenchyma tissue, 3-Sclerenchyma cells, 4-Schlerenchyma shealth, 5-Phloem, 6-Xylem and 7-Parenchyma cells

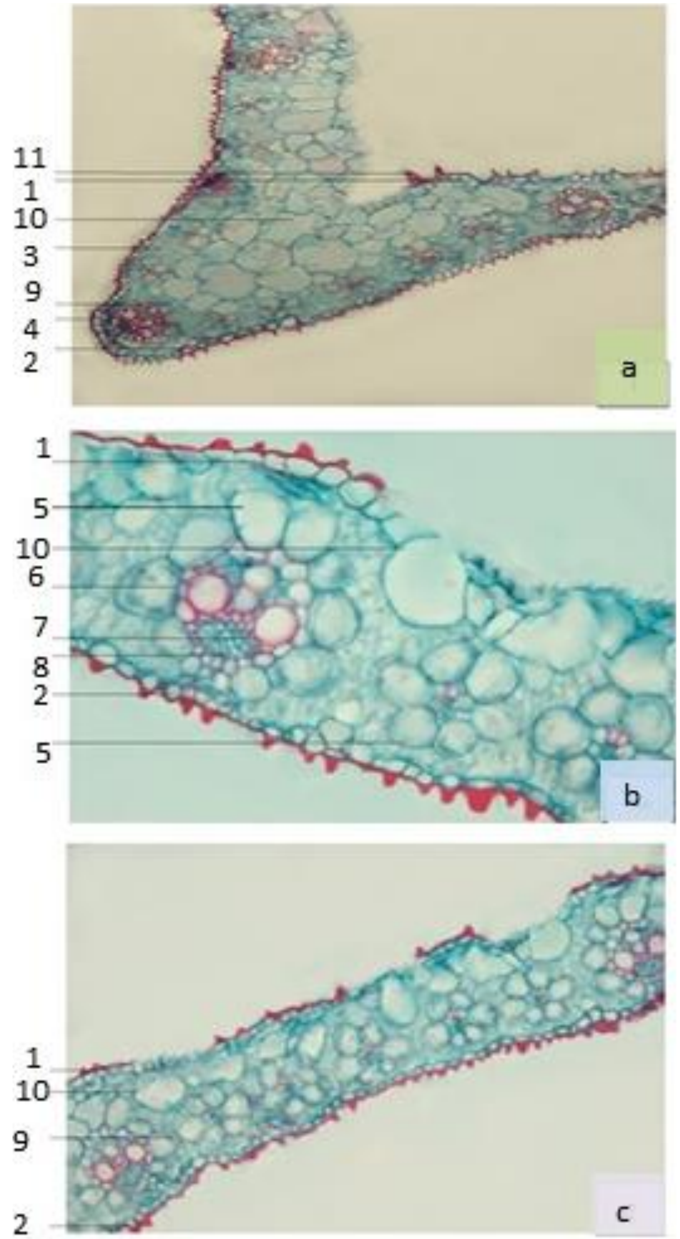

Figure 3. Cross sections of *C. barbata* SW. (leaf). a-Blade shape in leaf and coastal regions 200×, b-Vascular bundles in coastal regions 400×, c-Intercoastal region 200×, 1-Upper epidermis, 2-Lower epidermis, 3-Intercoastal region, 4-Coastal region, 5-Parenchyma shealth, 6-Schlerenchyma shealth, 7-Xylem, 8-Phloem, 9-Chlorenchyma tissue, 10-Bulliform cells and 11-Prickles.

and 7) indicate that the epidermis was covered with a thick cutin cuticle, which aids in water retention. The presence of stomata on both epidermal leaf surfaces, as well as the large intercellular spaces in leaf mesophyll tissue, facilitates gas exchange. Thin-walled bulliform cells with large vacuoles were plentiful in the upper epidermis above the prominent midrib region, and play a role in the folding or shrinking of the leaves when plants were under water stress, which reduces the amount of exposed leaf surface area and lowers rates of transpiration and light absorption. These results were consistent with the reports of Carmo-Silva et al. (2009), Gangwar et al. (2011) and Grigore and Toma (2017). In addition, the presence of different forms of silica cells, cork cells with suberized walls, and sclerenchyma cells in bundles that link with the epidermis via the large vascular bundles, were all traits that increase the strength and rigidity of the plants and provide protection against grazing herbivores.

The internal anatomical characteristics of the main vein leaves were very similar in *C. dactylon* (Figure 1a) and *C. barbata* (Figure 3a) and in both species ground tissues were composed of parenchyma and chlorenchyma cells. Vascular tissues consist of closed collateral vascular

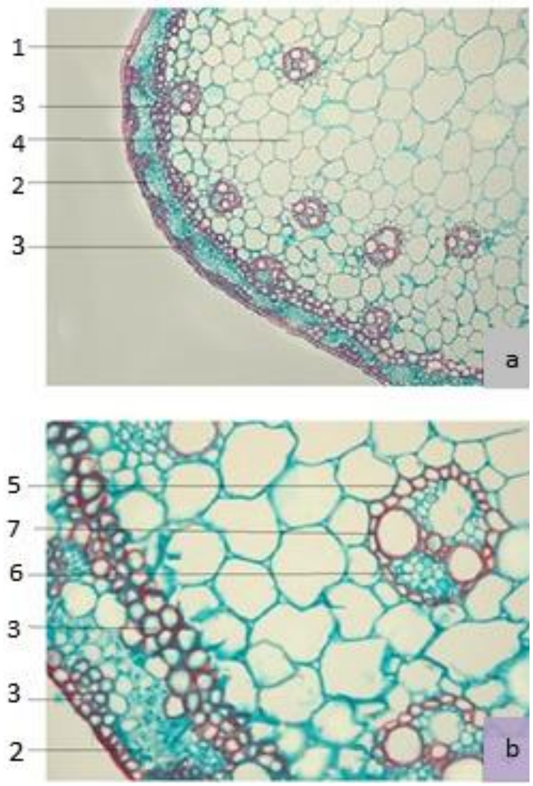

Figure 4. Cross sections in *C. barbata* SW. (Stem). a-100×, b-400×. 1-Epidermis, 2-Chlorenchyma tissue, 3-Sclerenchyma cells, 4-Parenchyma cells, 5-Sclerenchyma shealth, 6-Phloem, 7-Xylem.

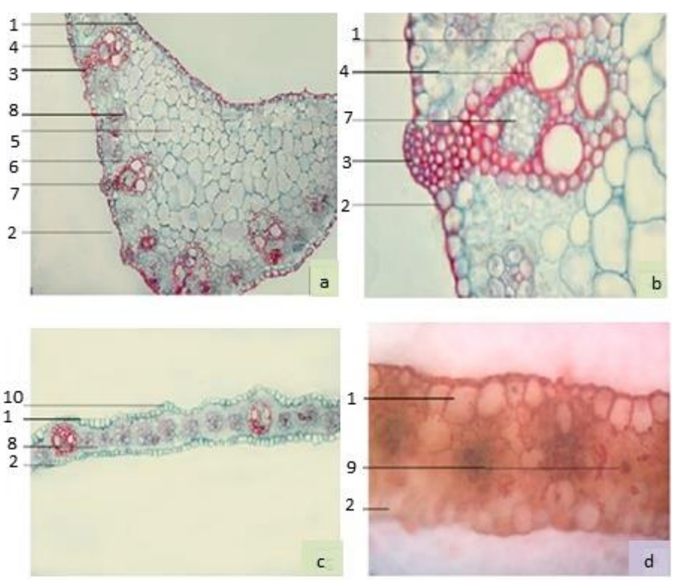

Figure 5. Cross sections in *S. verticillata* (L.) P. Beauv. (leaf). a-Blade shape in leaf and coastal regions 100×, b-Vascular bundles in coastal regions 400×, c-Intercoastal region 100×, d-Oil droplets in Intercoastal region 400×. 1-Upper epidermis, 2-Lower epidermis, 3-Sclerenchyma cells, 4-Sclerenchyma shealth, 5-Parenchyma cells, 6-Xylem, 7-Phloem, 8-Chlorenchyma tissue, 9-Oil drops, 10-Bulliform cells

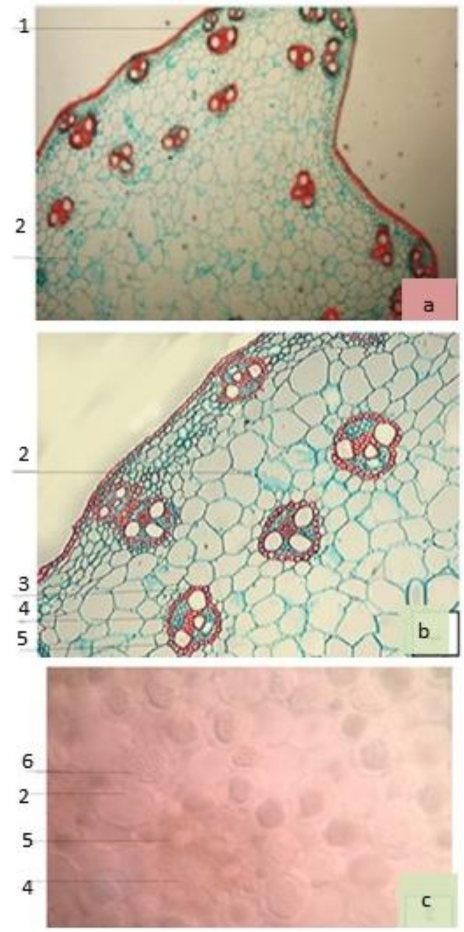

Figure 6. Cross sections of *S. verticullata* (L.) P. Beauv. (Stem). a, b-Parts of cross sections in stem, (a-50×, b-100×), c-Crystal stand in stem parenchyma tissue 200×. 1-Epidermis, 2-Parenchyma cells, 3-Sclerenchyma shealth, 4-Phloem, 5-Xylem and 6-Crystal sand.

bundles in an oval shape, with each bundle surrounded by two bundle sheaths composed of radially-shaped cells (chlorenchyma outside and sclerenchyma inside), whereas in *S. verticillata* (Figures 5a and b) and *P. coloratum* (Figures 7a and b), each vascular bundle was surrounded by a single bundle sheath composed of sclerenchyma cells and partially surrounded by chlorenchyma cells. In all four species, lateral vascular bundles were surrounded by a single bundle sheath of parenchyma cells, even though the four species belong to different genera and sub-families (*Chloridoideae* and *Panicoideae*).

Leaf blades contain a large number of vascular bundles, reflecting a greater ability to absorb water (Carmo-Silva et al., 2009), moreover, we observed oil droplets in the ground tissues of *P. coloratum* (Figure 5d) and *S. verticillata* (Figure 7d) leaves. Such oil droplets, which reduce water loss and can serve as an energy source

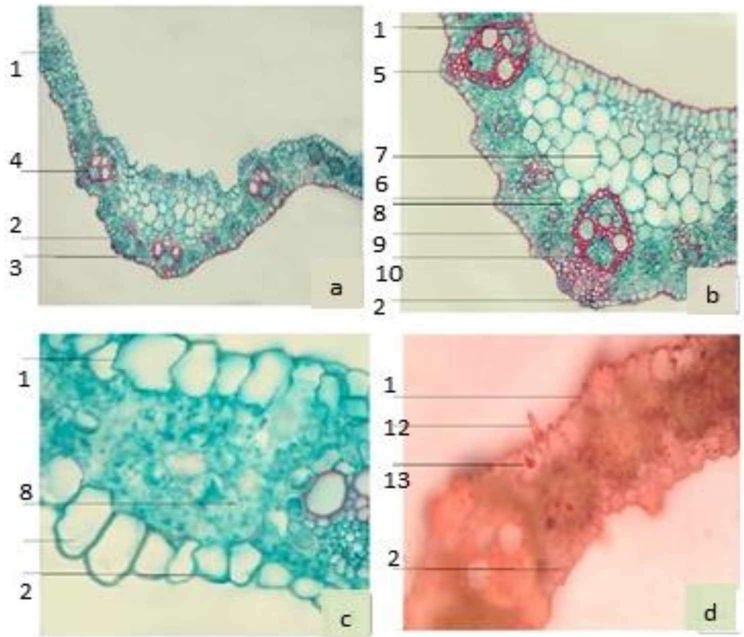

Figure 7. Cross sections in *P. coloratum* L. (leaf). a-Blade shape of leaf and coastal regions 100×, b-Coastal regions 200×, c-Intercoastal region 400×, d-Oil drops in an intercostal region 400×, 1-Upper epidermis, 2-Lower epidermis, 3-Coastal regions, 4-Intercostal region, 5-Sclerenchyma cells, 6-Sclerenchyma shealth, 7-Parenchyma cells, 8-Chlorenchyma tissue, 9-Xylem, 10-Phloem, 11-Bulliform cells, 12-Prickles and 13-Oil droplets.

(Eltahir and Abuereish, 2010), have been found in other epidermal cells of Poaceae species. Analysis of the stem cross-sections indicated (Figures 2, 4, 6, and 8) the presence of thick epidermal cellular walls due to an abundance of sclerenchyma tissue, which assists in the regulation of water loss, along with the presence of parenchyma cells that make up the ground tissue, which increases water storage capacities. We observed an abundance of vascular bundles in the ground tissues and metaxylem elements, which serve to enhance transport efficiency and provide maximal amounts of water and minerals to the vegetative parts of the plant for the proper functioning of photosynthesis and other biological processes. Moreover, the presence of many sieve tube elements in the phloem also improves nutrient transport (Hameed et al., 2010) and storage.

The results of our analysis indicate that the internal structures of the leaves and stems of these four plants exhibit a variety of adaptations that enhance drought tolerate capacity. These anatomical adaptations allow these species to thrive in the arid and semi-arid regions of Saudi Arabia, and can be used to increase vegetation cover and increase pasture area.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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