

INTERNAL STEM ANATOMY OF SIX *BROMUS* SPECIES GROWN IN DUHOK PROVINCE, KURDISTAN REGION-IRAQ

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ABSTRACT

The stems of six species of *Bromus* (*B. scoparius* L., *B. lanceolatus* Roth., *B. sterilis* L., *B. madritensis* L., *B. squarrosus* L. and *B. danthoniae* Trin.) which belongs to Poaceae family grown in Duhok province, Kurdistan region, Iraq were examined anatomically. The samples of *Bromus* species have been collected from Duhok city in spring 2022. The research was carried out at Biology department, Faculty of science, University of Zakho. The study aims to distinguish between the anatomical differences among the *Bromus* species since they share phenotypic traits that make it difficult to distinguish them apart. The samples were fixed in formalin- acetic-acid alcohol (FAA) and cross sections were prepared, examined using compound light microscope and photographed at different power. The highest number and measures of the vascular bundles, thicknesses of cuticle, epidermis, hypodermis, and the diameters of the minor and major vascular bundles (stele) were recorded in the *B. danthoniae*. while, the highest dimeters of metaxylem and cross section of stem were appeared in *B. scoparius* and *B. sterilis*, respectively. On the other hand, the lowest number and measures of the above-mentioned features were fluctuated among the *Bromus sterilis*, *B. lanceolatus*, *B. madritensis*. The cross sections indicated that there were valuable differences in both qualitative and quantitative values of the anatomical features among the species.

KEYWORDS: Annual Grass, Stem Anatomy, Stele, Vascular Bundle, Metaxylem.

1. INTRODUCTION

Poaceae or Gramineae is a large important diverse family that belongs to monocotyledonous flowering plants. It includes 780 genera and about 12,000 species (Christenhusz and Byng, 2016). According to the number of genera, it is the third largest family after Asteraceae and Orchidaceae, and the fifth one based on the species- wise after the Asteraceae, Orchidaceae, Leguminosae and Rubiaceae (Mehmood *et al.*, 2017). *Bromus* is a big genus of Poaceae family ranging between 100 and 150 species (Mabberley, 1997).

Grasses are found on almost every continent. They are considered as the most widely distributed and abundant groups of plants which constitute nearly 40.5% of the earth land, excluding Greenland and Antarctica (Reynold, 2016). Tropical and temperate grasslands constitute nearly 40 % of the Earth's terrestrial surface (White *et al.*, 2000). In addition, around 15% of monocots species diversity of the land surface are comprised of grass family (Clark, 2004). In southwest Europe, the tribe Bromine is only represented by the genus *Bromus*. Perennial and annual species can be found in this genus, and both life shapes are distributed throughout the genus (Acedo and Llamas, 2019) and they are native to temperate climates in both hemispheres (Pavlick, 1995).

The study by Mousa *et al.* (2021) included the cross sections of stems for eight species that belong to six genera of the Poaceae family. The results illustrated that the anatomical features of the stem had greatly contributed to separating the species depending on the diameter of the cross-section, the number of rows of sclerenchyma tissue, and the rows and number of vascular bundles, in addition to their different sizes and diameter of a single vessel.

Al-Khafaji and Al-Bermani (2019) studied the stem anatomy of six species of the genus *Poa* L. from Poaceae family in Iraq. Most of the anatomical features (diameter of stem cross section, sclerenchyma thickness, number and length of vascular bundles) indicated differences among species, which aided separate and isolate species within the genus.

El-Gawad and El-Amier (2017) found that the anatomical properties of *Arundo donax*, *Pennisetum setaceum* and *Saccharum spontaneum*, the three plants' stem have a typical monocot structure and the cross-section is more or less circular. In *P. setaceum* and *S. spontaneum* Ground tissue is not differentiated into cortex and pith, while *A. donax* has large hollow pith. The bundles sheath are sclerenchymatous tissues. The aim of the current study is to differentiate between the anatomical difference of the *Bromus* species which they have similar phenotypic features that make difficulties to distinguish between them.

2. MATERIAL AND METHODS

This research was carried out at Biology Department, Faculty of Science, University of Zakho, in spring 2022 during the growth season of *Bromus* species (annual grass). The samples of different species of *Bromus* (*B. scoparius* L., *B. lanceolatus* Roth., *B. sterilis* L., *B. madritensis* L., *B. squarrosus* L. and *B. danthoniae* Trin.) Poaceae family have been collected from many areas of Duhok city, Kurdistan Region, Iraq. All samples had the same size and age. The fresh samples of stem were fixed in (20 ml) of Formalin Acetic-acid Alcohol (FAA) for 24 hours, the solution of FAA was prepared by mixing 5ml of Formalin with 5ml of Glacial acetic-acid and 90ml of Alcohol (70%). The stem cross sections were stained with Fast Green and Safranin, and following that, they were placed in Canada Balsam for 24 hours (Al-Mukhtar *et al.*, 1982 and Brooks *et al.*, 1950). A magnifying Compound Light Microscope (Motic) with Camera adapter (Dino-Eye Microscope Eye-Piece Digital Camera (AM7025X) was used to measure and photograph the fixed slides, the average value of 25 observations for each quantitative feature had been measured which included the thickness of cuticle, epidermis and hypodermis and also the diameter of major stele, minor stele, metaxylem and pith. In addition to the number of vascular bundles for each row.

3. RESULTS AND DISCUSSION

The stem anatomical cross section in figure (1) of *Bromus* species shows that in general it has a spherical outline. The sections started with uniseriate outer layer of epidermal parenchymatous compact cells without free of intercellular spaces, covered with dissimilar thickness of cuticle waxy layer. Multiseriate layers of different shape of cells are located beneath the epidermis that represent the hypodermis which include continuous sclerenchyma tissues. The vascular bundles are of two rows, the small vascular bundles (minor) that located in the peripheral outer side near to the epidermis which are embedded in hypodermis and the second row of big vascular bundles (major) that embedded in ground tissues as shown in figure (2). These circular rows of vascular bundles make the pith clear and easy to be distinguished as a part of ground tissues. Closed conjoint collateral vascular bundle was recognized in all species stem sections that surrounded by sclerenchyma (fibers) tissue which represent the bundle sheath. Each vascular bundles contain primary xylem consisting of two relatively large sized of metaxylem that situated outward the pith and small protoxylem recognized near the central part of the sections closer to the pith. On the other hand, Shizogenous cavity (lacunae) was well developed beneath the protoxylem. Primary phloem was arranged and presented outside the metaxylem in the peripheral side. The pith is part of ground tissues that occupies the central part of stem made up of thin wall parenchyma cells.

Figure (1) shows the stem cross sections outlines with two shapes, spherical in *B. Scoparius*, *B. sterilis*, *B. madritensis* and *B. squarrosus* species and semispherical shape in *B. lanceolatus* and *B. danthoniae* with pyramidal girders. The epidermis is covered with cuticle layer in various thickness. The epidermis consists of single compact arrangement of parenchyma cells without intercellular spaces. The epidermis in some species was interrupted by patches of sclerenchyma tissues especially in *B. lanceolatus*, *B. sterilis*, *B. madritensis*, *B. danthoniae*. A few unicellular trichome appeared in species *B. danthoniae*.

The anatomical features in figure (2) illustrate that the hypodermis consists of well-developed continuous sclerenchyma tissues that expanded all over the peripheral section as shown in species *B. scoparius*, *B. sterilis*, *B. madritensis* and *B. squarrosus*. In contrast they are poorly developed and lignified narrow pyramidal girders shape of sclerenchyma tissues and separated by destroyed parenchyma layers in species *B. lanceolatus* and *B. danthoniae*. The vascular bundle has two metaxylem and many protoxylem. The vascular bundles are distributed in two circular rows of small bundles in peripheral side (minor) which is embedded in sclerenchyma tissues and big bundles in the middle side (major). The vascular bundles are surrounded by a sclerenchyma bundle sheath. The pith consists of parenchyma tissues and it is solid in *B. scoparius*, *B. squarrosus* and is occupied the central part of the stem in *B. danthoniae* and hollow pith in *B. lanceolatus*, *B. sterilis* and *B. madritensis*.

The results in table (1) illustrate that the highest number of major vascular bundles was (17) in whoever *B. sterilis* *B. madritensis*, *B. danthoniae* and the minor was (21) in *B. danthoniae*. While

the lowest number of major vascular bundles was (9) in *B. lanceolatus* and the minor was (8) in *B. scoparius*.

In addition, the highest mean thickness value of cuticle layer (3.46 μ m), epidermis (10.09 μ m) and hypodermis (94.24 μ m) which were ranged (2.57-4.90 μ m), (8.27-11.67 μ m) and (73.67-110.13 μ m) respectively, were recorded in *B. danthoniae* while the less thickened mean value of cuticle (2.19 μ m), epidermis (4.61 μ m) and hypodermis (33.47 μ m) with their ranges (1.44-2.84 μ m), (3.21-6.83 μ m) and (19.31-48.65 μ m) respectively were recorded in *B. sterilis*.

Moreover, *B. danthoniae* showed the highest mean diameters (99.71 μ m) of the major stele with the range of its maximum (114.61 μ m) and minimum value (89.98 μ m). While the minor stele records (55.59 μ m) with maximum range (78.37 μ m) and minimum (38.96 μ m), while *B. lanceolatus* recorded the lowest mean diameter (81.48 μ m) for the major stele (ranged between 58.67-100.36 μ m) and minor stele diameter (37.79 μ m) range between (25.80-50.57 μ m).

In contrast, the diameter of the metaxylem ranged between (21.66-33.33 μ m), that represent the highest mean value (27.85 μ m) in *B. scoparius*. Meanwhile, the lowest mean diameter (23.33 μ m) that ranged between (15.45-31.58 μ m) of metaxylem was documented in *B. madritensis*. On the other hand, the highest mean diameter of stem cross section was seen in *B. sterilis* and the lowest one was in *B. lanceolatus*, (1395.64 and 792.04 μ m respectively). Both the maximum and minimum range was reached between (1322.63-1482.70 μ m), and (752.61-832.51 μ m) respectively.

Plant anatomical and the morphological features are mostly influenced by both hereditary and environmental factors (Meng and Mao, 2013). Hence, the anatomical and morphological feature of plant gives an indication of the relationship between habitat and phylogenetic of plants (Cai and Guo, 1995 and Liu, 2006). The stem anatomical characteristics of the six *Bromus* species studied have revealed features, the cross sections of the stem are more or less spherical as well as having solid and hollow pith. The continuous and discontinuous cylinders of sclerenchyma tissues are near the periphery, and the small (minor) vascular bundles are embedded in these tissues. The major vascular bundles are embedded in the parenchyma of the ground tissues. This is equivalent to those mentioned by (Guo and Miao (2010) and Abd El-Gawad and El-Amier (2017).

Cuticle, epidermis and sclerenchyma hypodermis thicknesses and distribution, number of vascular bundles also the diameters of major and minor stele, metaxylem and cross section of stem showed differences among species which were a good anatomic and taxonomic value assisted to separate the species of the *Bromus* (Al-Khafaji and Al-Bermani, 2019). In order to improve the efficiency of the stele (major and minor) represented by the xylem and phloem, it is evident that the diameters of vascular bundles (major and minor) grow in length as the number of bundles reduces and decrease as the number of bundle increases (Abd El-Gawad and El-Amier, 2017). Similar results have been recorded by Al-Khafaji and Al-Bermani (2019) and Mousa *et al.* (2021). It has been observed that the mean diameter of metaxylem is directly proportional to the diameter cross-section of the stem, according to Cutler, *et al.*, (2007).

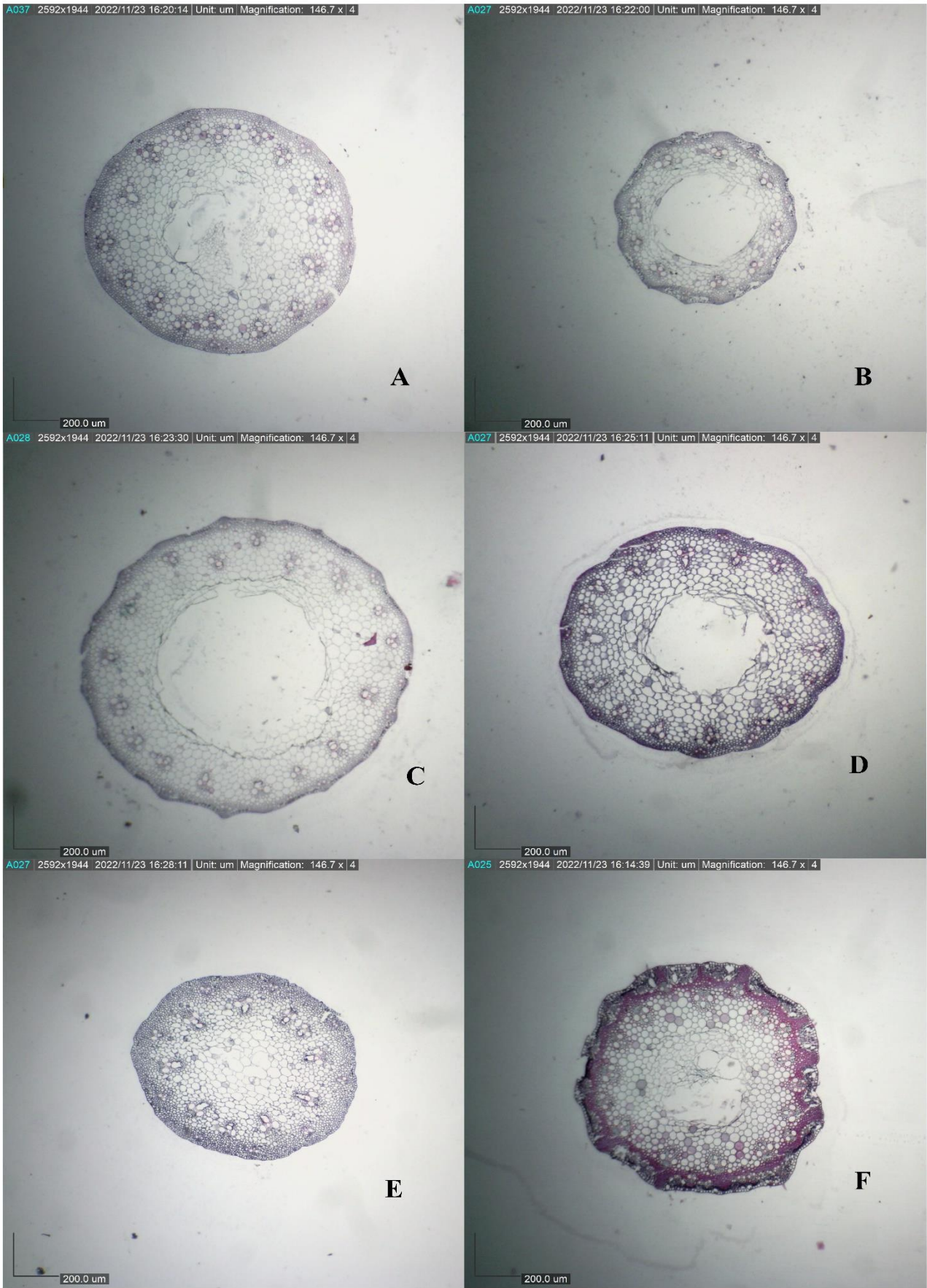


Figure (1): The stem cross sections of *B. scoparius* L. (A), *B. lanceolatus* Roth. (B), *B. sterilis* L. (C), *B. madritensis* L. (D), *B. squarrosus* L (E) and *B. danthoniae* Trin (F)

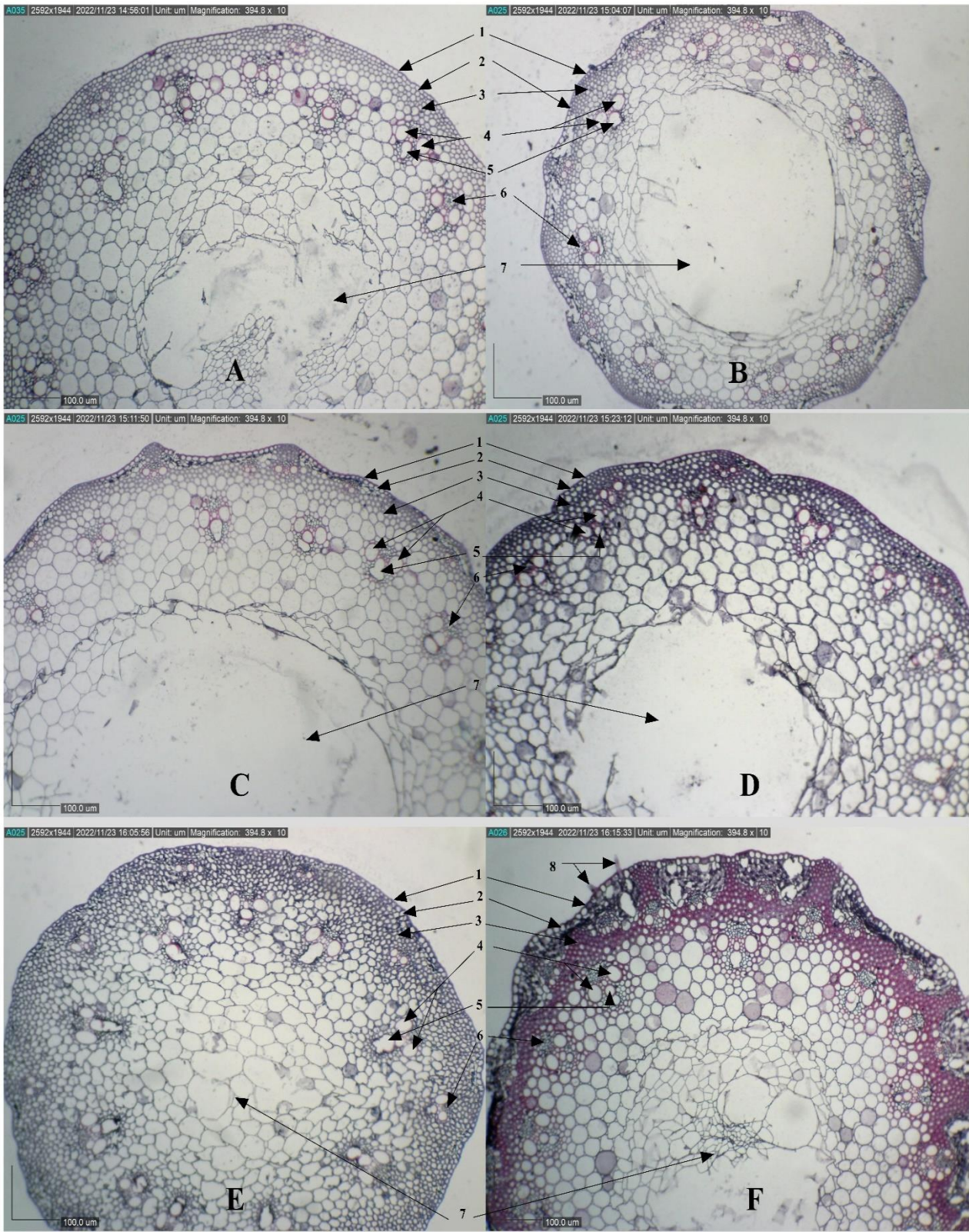


Figure (2): The stem cross sections of *B. scoparius* L. (A), *B. lanceolatus* Roth. (B), *B. sterilis* L. (C), *B. madritensis* L. (D), *B. squarrosus* L (E) and *B. danthoniae* Trin (F) showing: Cuticle=1, Epidermis=2, Hypodermis (Sclerenchyma tissues) =3, Metaxylem=4, Lacunae=5, Phloem=6, Pith=7, Trichome=8.

Table (1): The measurements feature of stem cross sections for studies species of *Bromus* *

| Features | Statistics | Species | | | | | |
|-------------------------------------|------------|-------------------------|---------------------------|------------------------|---------------------------|--------------------------|--------------------------|
| | | <i>Bromus scoparius</i> | <i>Bromus lanceolatus</i> | <i>Bromus sterilis</i> | <i>Bromus madritensis</i> | <i>Bromus squarrosus</i> | <i>Bromus danthoniae</i> |
| Number of vascular bundles | Major | 16 | 9 | 17 | 17 | 10 | 17 |
| | Minor | 8 | 9 | 20 | 10 | 9 | 21 |
| Cuticle thickness (µm) | Mean | 3.41 | 2.53 | 2.19 | 2.64 | 2.76 | 3.46 |
| | Range | 2.00-5.79 | 1.73-3.13 | 1.44-2.84 | 2.00-3.78 | 1.20-3.95 | 2.57-4.90 |
| Epidermis thickness (µm) | Mean | 6.86 | 6.37 | 4.61 | 4.86 | 5.63 | 10.09 |
| | Range | 4.88-8.27 | 4.05-9.00 | 3.21-6.83 | 3.61-6.47 | 3.63-8.19 | 8.27-11.67 |
| Hypodermis thickness (µm) | Mean | 46.98 | 47.09 | 33.47 | 41.65 | 59.42 | 94.24 |
| | Range | 31.75-65.78 | 30.98-64.38 | 19.31-48.65 | 28.74-58.18 | 39.90-74.94 | 73.67-110.13 |
| Diameter of Major stele (µm) | Mean | 96.11 | 81.48 | 87.51 | 87.21 | 91.34 | 99.71 |
| | Range | 84.06-126.11 | 58.67-100.36 | 68.79-103.26 | 69.80-121.08 | 67.14-118.44 | 89.98-114.61 |
| Diameter of minor stele (µm) | Mean | 49.02 | 37.79 | 44.38 | 45.20 | 50.93 | 55.59 |
| | Range | 30.96-69.00 | 25.80-50.57 | 23.88-63.23 | 34.41-65.23 | 36.85-65.28 | 38.96-78.37 |
| Diameter of metaxylem (µm) | Mean | 27.85 | 25.52 | 24.94 | 23.33 | 27.83 | 25.59 |
| | Range | 21.66-33.33 | 18.51-31.72 | 18.70-32.80 | 15.45-31.58 | 15.74-44.30 | 19.59-30.41 |
| Diameter of stem cross section (µm) | Mean | 1156.87 | 792.04 | 1395.64 | 1147.95 | 930.75 | 1091.60 |
| | Range | 1106.23-1212.35 | 752.61-832.51 | 1322.63-1482.70 | 1044.93-1248.52 | 829.11-1011.40 | 1041.94-1162.06 |

*The value in the table represents the average of 25 observations for each quantitative feature (µm= micrometer).

4. CONCLUSION

According to the current study it was concluded that both environmental and genetic factors affect the internal stem structure of *Bromus* species in variable parts of the stem contents. Moreover, this research showed that, since some anatomical features are identical to all species in the genus, other anatomical features vary between species and are valid for species identification and differentiation.

Finally, anatomical features might be seen as additional support for other systemic values even when they are not considerable enough to maintain the taxonomy of generic groups.

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