



RESEARCH ARTICLE

# Anatomical comparison of stems and leaves in local and introduced grape (*Vitis vinifera* L.) cultivars

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Received: 13 November 2025; Accepted: 15 January 2026; Available online: Version 1.0: 05 March 2026

**Cite this article:** Zaidan SA. Anatomical comparison of stems and leaves in local and introduced grape (*Vitis vinifera* L.) cultivars. Plant Science Today (Early Access). <https://doi.org/10.14719/pst.12715>

## Abstract

Grapes are economically significant and widely cultivated, with various types found in Iraq, including Ajami, Dis Anz, Halwani, Kamali, Al-Yaqut and Red Glabe. This study compares the anatomical structures of these six cultivars using microscopic techniques revealing distinct characteristics. Al-Yaqut exhibited the greatest thickness in peripheral epidermis, cuticle, vascular bundles and xylem vessels, while Des Anz showed the highest epidermal thickness in leaf blades. Kamali's upper epidermis walls were notably thicker. Stomata were anomocytic, with guard cells varying in shape. Crystal types included acicular, prismatic and star-shaped across different varieties. Overall, the study identified distinct anatomical variations among cultivars, which may hold functional and genetic significance for future research.

**Keywords:** characteristics; crystal types; cultivar identification; drought adaptation anatomy; leaf micromorphology; *Vitis vinifera*

## Introduction

*Vitis vinifera* L. is a perennial wood fruit crop that is utilized for winemaking, juice, dried fruits and alcoholic beverages (1). Grapes belong to the Vitaceae family. There are an estimated 8000 grape varieties that vary in size, color, shape, flavour and resistance to diseases and insects (2). Some grape varieties are seeded, others are seedless and there are black, red and white varieties (3). Grapes are widely cultivated worldwide and are highly important economically, with major production concentrated in the Mediterranean basin particularly in Italy, France, Spain and Greece while significant production also occurs in North and South America (notably the United States, Chile and Argentina), as well as in Australia, South Africa and parts of West and Central Asia, including Turkey and Iran, where climatic conditions are favorable for vine growth and productivity.

Studies have been conducted on numerous anatomical and functional aspects of grapevine parts (4–6). The growth process undergoes three distinct phases: 2 rapid growth periods separated by a delayed (ripening) phase, which results in water being transported to the fruit for enlargement. This rapid movement of water occurs across the stem and from xylem to phloem (7, 8). As the primary land plant organs, leaves play a key role in photosynthesis and their characteristics are closely linked to plant physiological functions (9). The number, function and environmental adaptation of stomata are influenced by cultivar type (10–12). Leaf anatomical traits are closely associated with a cultivar's ability to withstand environmental conditions. The anatomical characteristics of leaves include the density and size of stomata on the leaf epidermis, which are anatomical factors affecting plant growth

(13). Anatomical characteristics vary from one cultivar to another, determining the cultivar's ability to respond to environmental conditions (14–16) reported that the Grenache Noir cultivar outperformed the Syrah cultivar in leaf blade thickness, stomatal size and index and upper epidermis thickness. An anatomical study of the leaves of our red grape varieties grown under Mediterranean conditions, Aragonez, Cabernet Sauvignon, Syrah and Touriga Nacional, revealed significant differences between the varieties, Aragonez recorded the highest values for the studied traits, while Cabernet Sauvignon recorded the lowest values, while Syrah and Touriga Nacional recorded intermediate values (17). Previous findings showing the presence of anatomical differences between the grape varieties under study, as the shape of the leaf stalk and the midrib were anatomically different (18). Therefore, this study aims to identify the differences in anatomical structure between local and imported varieties and their effect on resistance to environmental stress.

## Materials and Methods

### Plant samples

Six cultivars of local and introduced grapes were used in this study. Samples were collected from Al-Hatimiyah area in Salah al-Din Governorate, as shown in the Table 1.

Samples of plants (leaves and stems) were collected from middle plant. After collecting them during the fruiting stage the plant samples (leaves and stems) were washed with distilled water, placed in sealed glass tubes filled with 70 % ethanol and brought to the lab in refrigerated containers.

**Table 1.** Grape cultivars used in the study, their locations and latitude and longitude lines

Cultivars	Location	Longitudes	Latitude
Red Glabe Ajami Dis Anz Halwani Kamali Yaqaot	Saladin - Al-Hatimiyya	44.4416136	33.9189009

### Sample preparation

The leaves and stems were cut from the middle of the stem and preserved in a formalin acetic acid alcohol (FAA) solution made (19). Following the 24 to 48 hr fixation period, the samples were kept in 70 % ethyl alcohol in accordance with previous procedures (20, 21). The plant stems were cut in half lengthwise to a length of 6 cm, resulting in the thinnest possible cross-section. The leaf blades and petioles were also cut into thin slices. The chlorophyll pigment was removed from the plant sections by placing them in a solution of 0.5 mL of artificial bleach and 1.0 mL of distilled water for 5 min. The sections were then stained with safranin for 1–2 hr and the excess pigment was removed by washing them with 70 % ethanol and then soaking them in 90 % ethyl alcohol. The slices were then placed in a 1:1 solution of xylene and absolute alcohol for 2 min. Finally, the slices were transferred for examination using a KRUSS compound microscope and an AmScope Model MU1000 camera mounted on the microscope was used to capture images.

### Epidermal peel preparation

The leaf epidermis was scraped midway between the base and the apex using a sharp dissecting scalpel. Following scraping, the sample was washed with distilled water and left for 5 min in a Petri dish with 5 % sodium hypochlorite (artificial bleach). It was cleaned with distilled water and put in a different Petri dish with 10 % KOH for 5 to 10 min. After that, 70 % ethyl alcohol was added to the solution for 10 to 15 min. Then, the sample was placed in a Petri dish with 1 % safranin stain and left for 30 to 45 min. Following a distilled water wash, the sections have been subjected to an ascending series of 70, 95 and 100 % ethyl alcohol, respectively. It was positioned for 10 min at each concentration. After that, it was placed in a Petri dish with xylene for 10 min. It was after that prepared for microscopic examination by being put on a slide with a drop of water and drop of xylene and covered with coverslip (21, 22).

### Microscopic examination

The test was performed using an AmScope camera (model MU1000) mounted on a KRUSS compound microscope. The stoma index was calculated according to the following equation (23):

Stomatal Index =

$$\frac{\text{Number of stomata}}{\text{Number of stomata} + \text{Number of normal epidermal cells}} \times 100$$

The results were analyzed statistically according to the randomized complete block design (RCBD) and the means were compared with the use of the least significant difference (LSD) at a significant level of 0.05 using the Genstat program (24).

## Results and Discussion

### Cross-sectional study of the stem

The results showed that the cross-section of the stems of all varieties was circular and that there were significant differences between grape cultivars in all the traits in the stems studied (Table 2). The stem cross-section comprised the following tissues:

#### Epidermis

Since stem growth is secondary, the epidermis is replaced by the periderm, which consists of a single layer of cells in all cultivars. The shape of the epidermal cell was oval to polygonal, interspersed with lenticels and hairs. The maximum thickness of the peripheral epidermis reached 44.50  $\mu\text{m}$  in the Yakut cultivar and the minimum was 29.50  $\mu\text{m}$  in the Red Glabe variety. The epidermis layer was covered from above by the cuticle layer, which reached a maximum thickness of 5.61  $\mu\text{m}$  in the Yakut variety and a minimum thickness of 3.91  $\mu\text{m}$  in the Red glabe cultivar (Table 2).

#### Cortex

The cortex consisted of two types of tissues: lamellar collenchyma cells, in which the thickness of the primary wall is limited to the inner and outer tangential walls, while the radial walls remain thin and these thickenings are in the form of layers or sheets stacked on top of each other. This is followed by the second layer, which consists of parenchyma cells of the chlorenchyma type. The highest average thickness of the cortex is 67.1  $\mu\text{m}$  in the Halwani cultivar and the lowest is 48.5  $\mu\text{m}$  in the Ajami cultivar (Table 2).

#### Vascular cylinder

The vascular cylinder is composed of secondary xylem and secondary phloem. The vascular cylinder is externally surrounded by secondary pericyclic fibers, which are sclerenchyma tissue that provide support to the vascular bundles. The phloem occupies the outer position, while the xylem is located internally. This arrangement reflects the early stage of secondary growth in the stem. The first annual ring has already formed in all varieties, which makes it difficult to count and distinguish vascular bundles in cross-section. Xylem vessels of various diameters and of the ring-porous pattern (ring-shaped xylem) have also been observed. The highest average thickness of the vascular bundle was 320.53  $\mu\text{m}$  in the

**Table 2.** Quantitative characteristics measured by  $\mu\text{m}$  for stem cells of the studied cultivar

Cultivars	Thickness of epidermal layer	Cuticle thickness	Cortex thickness	Vascular bundle thickness	Vessel element diameter
Red Glabe	29.50	3.91	59.53	211.56	14.20
Ajami	33.46	5.10	48.50	169.46	13.50
Dis Anz	32.50	4.35	43.56	155.50	16.16
Halwani	31.46	4.11	67.16	224.33	17.20
Kamali	34.43	4.92	52.50	300.56	18.50
Yaqaot	44.50	5.61	62.60	320.53	19.36

\* The numbers represent the average of ten replicates of grape cultivars at the probability level  $p \leq 0.05$ .

Yaqut cultivar and the lowest average was 155.50  $\mu\text{m}$  in the Dis Anz cultivar. The highest average diameter of the xylem vessel was 19.36  $\mu\text{m}$  in the Yaqut cultivar and the lowest average was 13.50  $\mu\text{m}$  in the Ajami cultivar (Fig. 1; Table 2).

### Pith

In all cultivars, the pith consists of ordinary parenchyma cells, equilateral and interspersed with ordinary schizogenous intercellular spaces.

The increase in the thickness of the epidermis, cortex, vascular bundle thickness and xylem vessel diameter is an adaptation that helps the plant resist environmental conditions such as salinity (25).

### Study of cross-sections of leaf blade cells

The cross-section of green leaves consists of the following layers as shown in Fig. 2.

#### Epidermis

The epidermis in all cultivars consists of a single layer of oval to elongated cells and the upper epidermis has larger cells than the lower epidermis. The highest average thickness of the upper epidermis was observed 57.33  $\mu\text{m}$  in the Des Anz cultivar and the lowest average reaches 34.40  $\mu\text{m}$  in the Kamali cultivar. The highest average thickness of the lower epidermis reaches 54.26  $\mu\text{m}$  in the Des Anz cultivar and the lowest average is 36.26  $\mu\text{m}$  in the Kamali cultivar (Table 3).

#### Mesophyll tissue in the leaf

This consists of 2 mesophyll layers: a palisade layer at the top and a spongy layer at the bottom in all species (Fig. 2). The columnar layer is composed of two rows of elongated columnar cells, while the spongy layer is composed of polygonal cells. The highest average thickness of the columnar layer was 60.60  $\mu\text{m}$  in the Des Anz cultivar and the lowest was 34.60  $\mu\text{m}$  in the Red Glabe cultivar (Table 3). As for the spongy layer, its highest thickness was 58.43  $\mu\text{m}$  in the Yaqout cultivar and its lowest rate was 37.46  $\mu\text{m}$  in the Red Glabe cultivar, which indicates that there are significant differences between grape cultivars (Table 3). The results in Table 3 show that there is a variation in the parts of the leaf blade in the cross section and that this variation may increase the number of sites available for absorbing carbon dioxide per unit area of the leaf surface (26) and may help in maintaining the rates of photosynthesis for some time if stomata are closed in order to conserve water (27), which makes the Des Anza cultivar more adapted to drought as a result of the anatomical adaptations present on the leaves that may be associated with water stress. The cross-section in the midrib region consists of the following layers (Fig. 2):

#### Epidermis

It consists of a single layer of oval to polygonal cells in all cultivars. The epidermis is permeated by stomata and adenoid hairs are

found only in the Agamic cultivars. It consists of a single layer of oval to polygonal cells in all cultivars. The epidermis is permeated by stomata and adenoid hairs are found only in the Agamic cultivars. The adenoid hairs are characterized by being multicellular and uniseriate. This is considered a distinctive feature of cultivars. The adenoid hairs are characterized by being multicellular and uniseriate.

#### Cortex

The cortex in the midrib consists of ordinary parenchyma cells separated by ordinary schizogenous intercellular space. Oil droplets are also distributed in the cortex region of the midrib of the leaf in the cultivar Kamali, Fig. 2. This is considered a distinctive feature of the cultivar.

#### Vascular bundle

The vascular bundle in the studied cultivars is oval-shaped and divided into several bundles, the number of which varied among the different cultivars. They can be divided into groups:

- **The first group:** The vascular bundle in the form of 9 bundles in the Red Glabe and Ajami cultivars, where the large bundle was in the middle and the rest of the bundles were successive in size to form an oval shape in the Red Glabe cultivar and in the form of 2 bundles in the middle and the rest of the bundles were successive in size in the Ajami cultivar.
- **The second group:** The vascular bundle, numbering 12 in the 2 cultivars, Dis Anz and Yaqoot, in the form of 2 bundles in the middle and the remaining bundles follow one another to form an oval shape in both cultivars.
- **The third group:** The vascular bundle consists of 13 bundles in Halwani, in the form of 2 bundles in the middle and the remaining bundles follow one another to form an oval shape.
- **The fourth group:** The vascular bundle consists of 14 bundles in the Kamali, with 2 bundles in the middle and the remaining bundles follow one another to form an oval shape.

In dicots, xylem is inside, phloem outside. In all cultivars, the vascular bundle is surrounded by a parenchymatous bundle sheath. The results (Table 3) showed significant differences in all studied characteristics of the mesophyll cells, except for cuticle thickness. Similar results were obtained earlier (28).

### Cross-sectional study of the petiole

Results of the study showed that the cross-sectional shapes of the leaf petiole were elongated ovate in all cultivars, with significant differences between grape cultivars in the studied traits except for the thickness of the cuticle (Table 4). The section consisted of the following layers:

#### Epidermis

The epidermis consists of a single layer of cells in all cultivars. The shape of the epidermal cell was oval to polygonal, with the highest

**Table 3.** Quantitative characteristics measured (in  $\mu\text{m}$ ) for the leaf blade cells of the studied cultivars

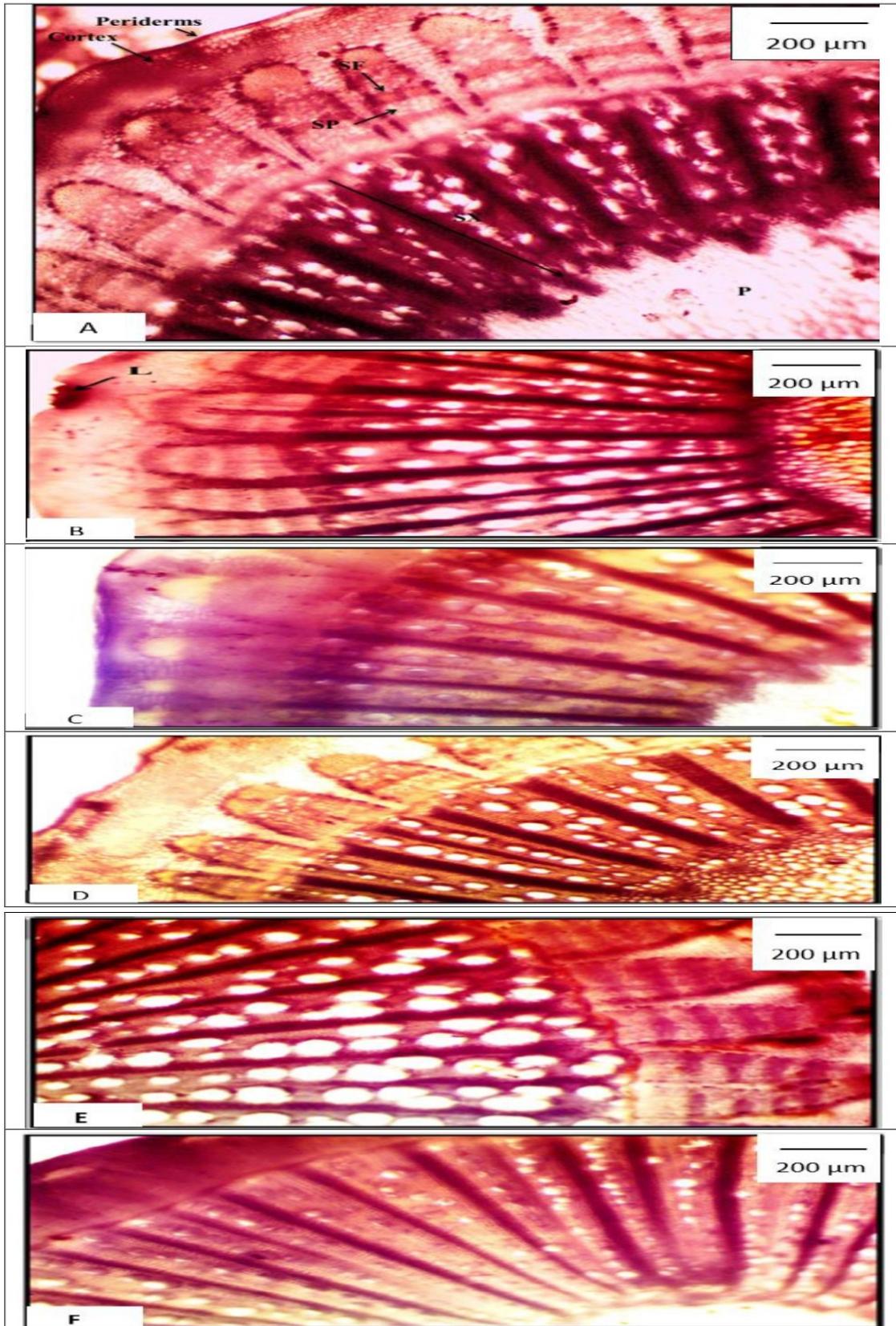
Cultivars	Upper epidermis thickness	Lower epidermis thickness	Number of columnar cell rows	Thickness of the columnar layer	Thick spongy layer
Red glabe	45.36	38.53	2	34.60	37.46
Ajami	44.46	50.46	2	41.53	45.60
Dis Anz	57.33	54.26	2	60.60	40.13
Halwani	39.53	39.53	2	45.66	44.36
Kamali	34.40	36.26	2	50.46	46.36
Yaqoot	39.56	44.20	2	57.73	58.43

\* The numbers represent the average of ten replicates of grape cultivars at the probability level  $p \leq 0.05$ .

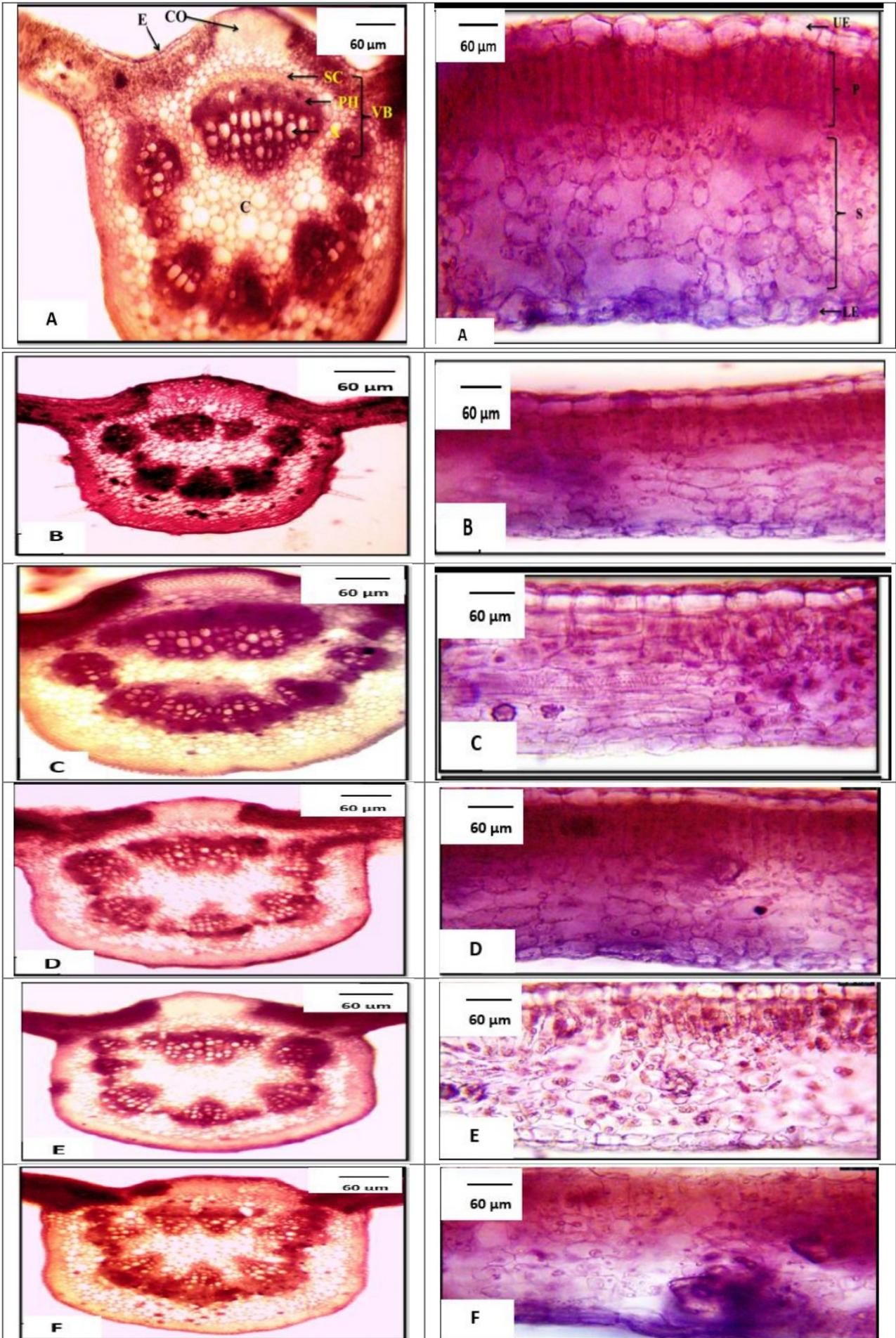
**Table 4.** Quantitative anatomical characteristics (in  $\mu\text{m}$ ) of the leaf stalk cells of the studied cultivars

Cultivars	Cuticle thickness	Epidermis thickness	Cortex thickness	Number of vascular bundles
Red Glabe	2.80	27.50	60.46	20
Ajami	4.10	29.30	66.53	23
Dis Anz	3.30	40.20	80.30	33
Halwani	3.50	42.20	74.36	24
Kamali	3.60	50.23	69.36	20
Yaqoot	4.30	39.33	77.33	27

\* The numbers represent the average of ten replicates of grape cultivars at the probability level  $p \leq 0.05$ .



**Fig. 1.** A cross-section of the stem for 6 local and introduced grape cultivars. A- Red Glabe; B-Ajami; C-Des Anz; D-Halwani; E-Kamali; F-Yaqut. SF = Secondary fiber, SP = Secondary phloem, SX = Secondary xylem, P = Pith.



**Fig. 2.** A cross section of the leaves of 6 local and introduced grape cultivars showing the layers of the leaf blade and the midrib. A- Red Glabe; B- Ajami; C- Des Anz, D- Halwani, E- Kamali, F- Yaqut. e = epidermis, CO = collenchyma, Sc = sclerenchyma, ph = phloem, x = xylem, vb = vascular bundle, UE = upper epidermis, LE = lower epidermis, P = palisade layer, S = spongy layer.

average thickness of the epidermis reaching 50.23  $\mu\text{m}$  in the Kamali cultivar and the lowest of 27.50  $\mu\text{m}$  in the Red Glabe cultivar. The epidermis layer was covered from above by the cuticle, which reached a maximum thickness of 4.30  $\mu\text{m}$  in the Yaqout cultivar and a minimum of 2.80 in the Red Glabe cultivar (Table 4).

#### Cortex

The cortex in the cross section consisted of 2 cell types: angular collenchyma cells located just below the epidermis and consisting of a single layer of cells, followed by a second layer composed of parenchyma cells of the chlorenchyma type. The highest average cortical thickness was 80.30  $\mu\text{m}$  in the Des Anz cultivar and the lowest was 60.46  $\mu\text{m}$  in the Red Glabe cultivar (Table 4).

#### Vascular cylinder

The vascular cylinder consists of the phloem to the outside and the xylem to the inside. The vascular bundle was elongated cordiform in all cultivars. The vascular bundle in the studied cultivars was oval-shaped and divided into several bundles, the number of

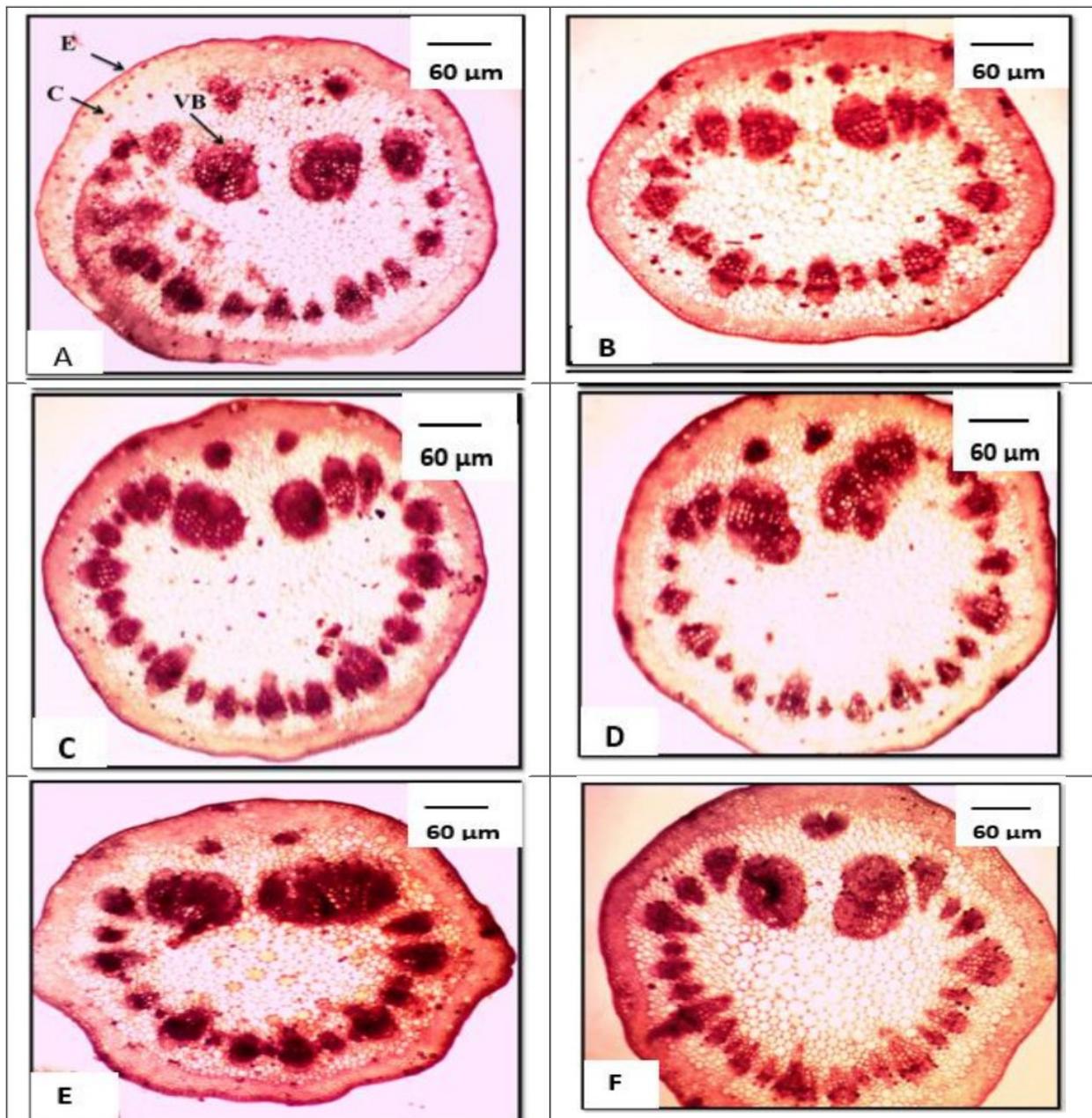
which varied among the different cultivars. They could be divided into groups, as shown in Fig. 3.

- **The first group:** The vascular bundle in the form of 9 bundles in the Red Glabe and Ajami cultivars, where the large bundle was in the middle and the rest of the bundles were successive in size to form an oval shape in the Red Glabe cultivar and in the form of 2 bundles in the middle and the rest of the bundles were successive in size in the Ajami cultivar.

- **The second group:** The vascular bundles number 12 in the 2 cultivars, Dis Anz and Yaqoot and they form 2 bundles in the middle and the remaining bundles follow one another to form an oval shape in both cultivars.

- **The third group:** The vascular bundle consists of 13 bundles in the confectioner's batter, in the form of 2 bundles in the middle and the remaining bundles follow one another to form an oval shape.

- **The fourth group:** The vascular bundle consists of 14 bundles in the perfect form, with 2 bundles in the middle and the remaining bundles follow one another to form an oval shape.



**Fig. 3.** A cross section of the leaf stalk of six local and introduced grape cultivars. A- Red Glabe; B- Ajami; C- Des Anz; D- Halwani; E- Kamali; F- Yaqout. E= epidermis, C= cortex, VB= vascular bundle.

The vascular bundle consists of the phloem to the inside and the xylem to the outside. The vascular bundle is surrounded in all cultivars by a parenchymatous bundle sheath.

### Study of the epidermis in green leaves

The results showed that the anticlinal walls differed in the surface view of the epidermal cells in grape cultivars, as well as in the upper and lower surface of the leaf, both adaxial and abaxial, for the same plant cultivars. The cell walls of the upper epidermis were characterized by being straight for all cultivars. The walls of the Kamali cultivar were very thick compared to the other cultivars, which is considered a distinctive feature of the cultivars, while the cell walls of the lower epidermis were characterized by being semi-wavy in all cultivars as well (Fig. 4). The stomata were distributed only on the surface of the lower epidermis (hypostomatic type), meaning that the stomata are present only on the lower surface, of the anomocytic type, consisting of 6 normal epidermal cells surrounded by hexacytic cells in all cultivars. Table 5 shows significant differences between grape cultivars in stomatal length, width and stomatal index. The density of stomata on the lower epidermis is represented by the stomatal index. The lowest stomatal index on the lower surface was in the Halwani and Kamali cultivars, which had a stomatal index of 16, followed by Ajami and Yakut cultivars, which had a stomatal index of 20. The stomatal index of the other cultivars was equal, reaching a maximum of 25.

Regarding stomatal dimensions, the minimum average stomatal length on the lower surface was 48.30  $\mu\text{m}$  in the Des Anza cultivar and 64.63  $\mu\text{m}$  in the Halwani cultivar. The other cultivars showed overlap in this trait (Table 5). The shape of the guard cells on the lower surface was reniform in all cultivars, ranging from short, plump reniforms to elongated, narrow reniforms. Guard cells on the lower epidermal surface were characterized by their short, plump reniform shape in the Red Glabe and Des Anza cultivars, while they were elongated and plump reniforms in the remaining cultivars.

The results (Table 5) showed variations in anatomical characteristics among grape cultivars, which may be related to drought resistance (29) or may be due to differences in the growth stage and environmental conditions (30). Stomata size appears to be a genetically determined trait and may also be influenced by environmental and physiological characteristics. The stomatal index is a direct measure of the proportion of cells that have differentiated into stomata, which can be altered by environmental factors (30, 31). The Des Anz cultivar exhibited the smallest stomatal size compared with the other studied cultivars. Smaller stomata are associated with reduced water loss and more controlled gas exchange, which may contribute to greater tolerance to environmental conditions and lower disease susceptibility (32–34).

Crystals of different shapes were distributed in the upper epidermal cells of the leaf, as they were of the raphides crystals type or needle crystals in the Red Glabe, Des Anz and Halwani cultivars

and of the Prismatic crystals in the Ajami cultivar and star-shaped druses crystals in the Des Anz cultivar. These are considered distinctive and taxonomic characteristics for comparison between the cultivars. The results in Fig. 5 indicate a variation in the shapes of the crystals in the studied cultivars. The function of these crystals is not entirely clear, but research results indicate that they may participate in regulating calcium in tissues, detoxifying heavy metals and resisting drought (35). The increase in crystals in grape leaves helps resist water stress conditions. The crystals also serve as a source of carboxyl groups that can be converted to CO by the enzyme oxalate oxidase during times of low CO or when the stomata close due to water stress.

### Conclusion

Anatomical differences were found among the grape cultivars Red Glabe, Ajami, Kamali, Dis Anz, Halwani and Yaqoot. The results showed that Yaqoot recorded the highest values for the stem anatomical traits of the stem, while Dis Anz recorded the highest values for the average thickness of the upper and lower epidermis and the sclerenchymatous tissue. Anatomical differences were detected among the grape cultivars studied. Yaqoot showed the highest stem anatomical values, Dis Anz exhibited the greatest epidermal and sclerenchymatous thickness, non-glandular hairs were observed only in Ajami, Kamali had thicker adaxial epidermal walls and all cultivars showed anomocytic stomata. The results of this study are important for understanding the anatomical structure of the plant parts of grape varieties and for studying the relationship between local and imported grapes, which demonstrates the importance of variation in anatomical structure for resisting environmental stresses. Further studies on grapes from a genetic perspective are necessary to determine the degree of relatedness between them and the role of genes in resisting environmental stresses.

### Compliance with ethical standards

**Conflict of interest:** Author does not have any conflicts of interest to declare.

**Ethical issues:** None

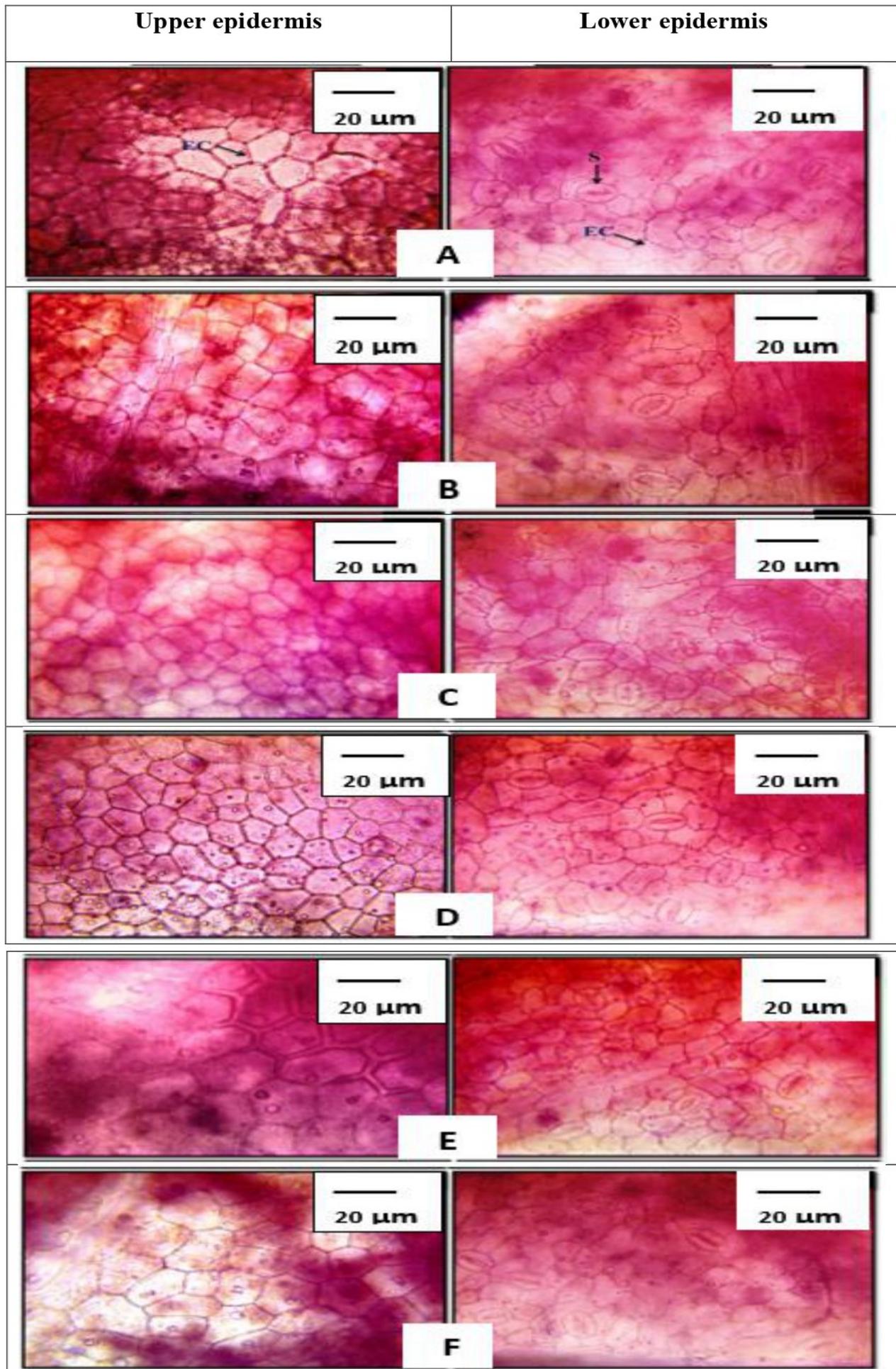
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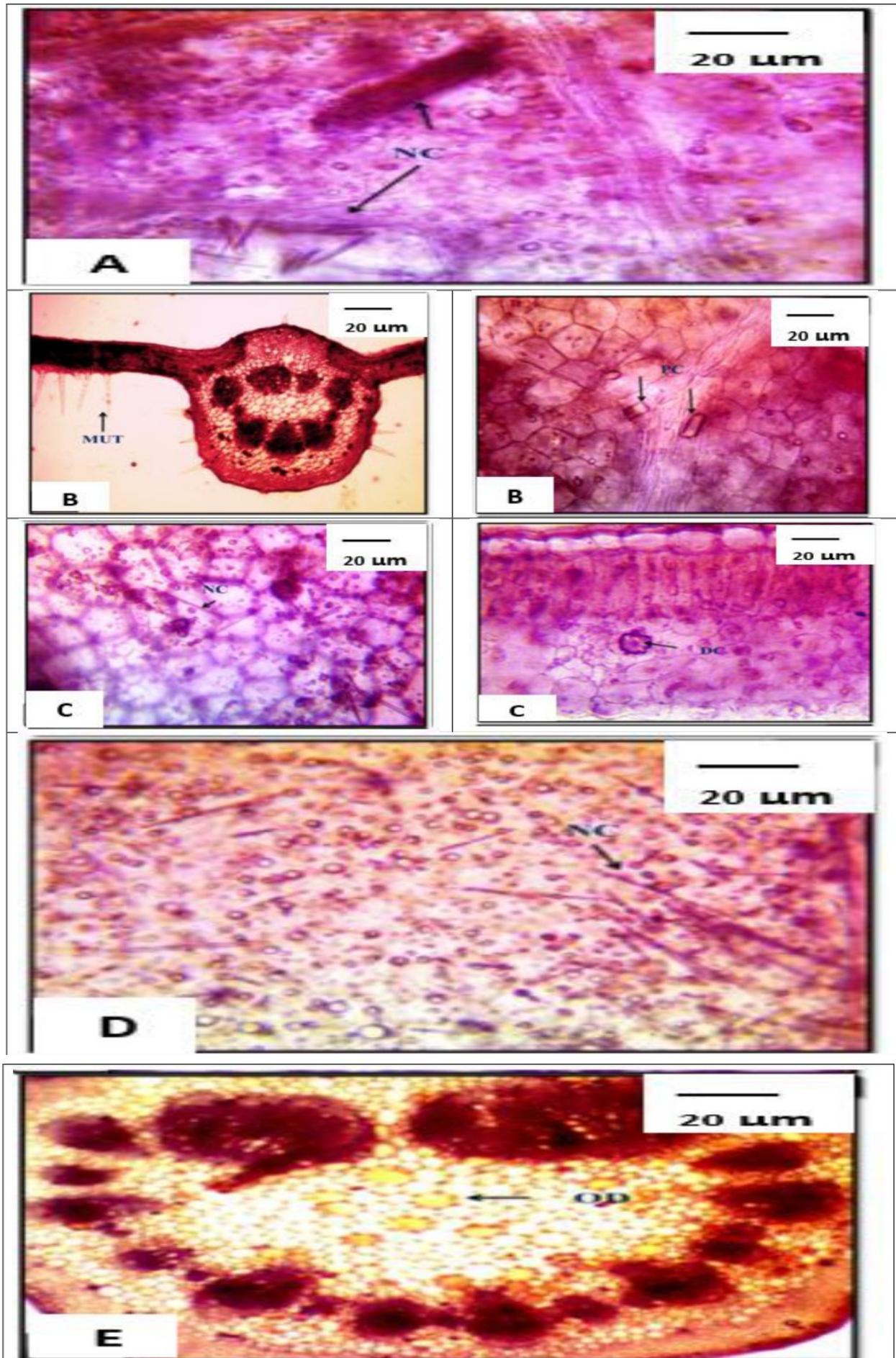
**Table 5.** Quantitative characteristics (in  $\mu\text{m}$ ) of the stomatal complexes in the leaf epidermis of the studied cultivars

Cultivars	Lower skin		
	Length of the stoma	Stoma width	Stomatal index
Red glabe	50.53	53.73	25
Ajami	52.43	55.56	20
Des Anz	48.30	42.43	25
Halwani	64.63	46.50	16
Kamali	50.46	56.43	16
Yaqoot	60.63	66.63	20

\* The numbers represent the average of ten replicates of grape cultivars at the probability level  $p \leq 0.05$ .



**Fig. 4.** A section showing the structure of the epidermis in the green leaves of 6 cultivars of local and introduced grapes. A- Red Glabe; B- Ajami; C- Des Anz; D- Halwani; E- Kamali; F- Yaqt. S = Stomata, EC = Epidermal cells.



**Fig. 5.** Distribution of crystalline structures and other structures in the skin of 6 cultivars of local and introduced grapes. A- Red Glabe; B- Ajami; C- Des Anz; D- Halwani; E- Kamali; F- Yaqt. NC = Needle crystal, PC = Prismatic crystal, DC = Druces crystal, OD = Oil droplets, MUT = Multicellular uniserait trichomes.

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