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Study of the upper epidermis of leaves of *Zizyphus* species and varieties in Central and Southern Iraq

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Abstract

The results showed that some anatomical features of the leaves have good taxonomic value for distinguishing between these species and varieties. The upper epidermal cells of the studied species and varieties showed clear differences in their dimensions. Although the two species *Z. mauritiana* and *Z. spina-christi,* existed under the same environmental conditions, the dimensions of the upper epidermal cells of the first species were more significant than those of the second, which made them a distinctive feature of this species. *Z. nummularia,* the upper epidermis of the varieties of this species, was also significant in dimensions, unlike *Z. nummularia*, in which the upper epidermal cells were large in dimensions. The difference was also evident in the thickness of the surface walls of the upper epidermal cells, which were thicker in most species of *Z. mauritiana* than in the rest of the species, especially the cells of Armouti Tailandy, which were even thicker than the walls of the upper epidermal cells of *Z. nummularia*, despite the harsh conditions in which this species lives. The variation in the distribution of stomata on the surface of the upper epidermis was apparent between species and sometimes between varieties of the same species. As mentioned in the results, this variation helped include the species and varieties in one group. This reflects the close evolutionary relationship between them. Then, the frequency of stomata on the surface of the upper epidermis of species *Z. nummularia* is higher than that of the rest of the species. This may seem natural for the species *Z. nummularia* due to the nature of the desert environment.

Keywords

Rhamnaceae; *Z. jujuba*; *Z. mauritiana* ; *Z. nummularia; Z.spina-christi*; *Ziziphus*

Introduction

The genus *Ziziphus* belongs to the Sidr family, Rhamnaceae. This family is one of the large plant families, including approximately 55 genera and approximately 950 species. The genus *Ziziphus* is one of the most prominent genera of the Sidr family, as the total number of species belonging to this genus is approximately 170 species, which are widespread in the tropical, subtropical, and warm temperate regions of the world (1).

Recently, many people have received significant attention from *Ziziphus* trees due to their ability to withstand drought conditions and wide adaptation to different soils (2). *Ziziphus* trees have been used for medicinal purposes and physical therapy, and some previous studies indicated that some species of the genus *Ziziphus*, including the species *Z. Spina-christi*, are beneficial for controlling liver and intestinal disorders and treating skin diseases. It is also used as antiseptics and other benefits that have made it the focus of attention of scholars (3).

The *Ziziphus* plant is of special importance, as it is one of the plants of Paradise mentioned in the Holy Quran and it has economic and medicinal importance and many benefits. *Ziziphus* leaves have an incredible and attractive colour and are consumed on a wide commercial scale. Their fruits are used in the food industry. The *Ziziphus* family is highly nutritional because it contains sugars, proteins, organic acids, amino acids, vitamins, fats, fibre, mineral salts and antioxidants (4).

Interest in growing *Ziziphus* has increased globally because it is an unexploited fruit tree that tolerates harsh environmental conditions. *Ziziphus* trees can grow in soils unsuitable for growing other fruit trees due to the ability of tall trees to withstand drought conditions and poor soils, and they also tolerate high temperatures, which may reach 50 degrees Celsius and low temperatures (5). Therefore, its cultivation is widespread in dry and semi-arid regions worldwide. *Ziziphus* tree cultivation is widespread in tropical Africa, northern Australia, the Pacific Islands, and the Caribbean Islands. In recent years, it has been grown in southern European countries, such as Spain and Italy (6). As for Iraq, *Ziziphus* cultivation is widespread in the south and central regions, which contain many agricultural varieties of the *Ziziphus* plant, including the apple variety, which is considered one of the most essential agricultural varieties in Iraq due to its significant economic importance represented by its high and early productivity and its good financial return, which has increased farmers' interest in it in recent years (7).

Due to the importance and scarcity of anatomical studies on plants under the environmental conditions of the central and southern regions of Iraq, the current research focused on investigating the following axes: Studying the anatomical characteristics of the vegetative organs represented by studying the upper epidermis of the leaves *Ziziphus*.

Materials and Methods

Plant specimens collection

The current study relied on fresh samples collected in field trips to the Al-Kaziza area in Basra, Al-Aziziya, Baghdad, and Al -Najaf Governorate. Five species and five varieties of the Rhamnaceae family Table 1 were selected from (17/10/2022). Until (29/4/2023), samples of the samples were deposited in the herbarium of the College of Education for Girls / University of Kufa after information was recorded on them, including the name of the sample, the date of collection, the name of the mosque, and the environment of the area from which it was collected.

Preparing the used dye

The dye used when examining samples is safranin-glycerin, which was prepared by adding one volume of safranin dye to six volumes of glycerin (6:1). It was used to facilitate the movement of the slide cover during examination and then ease the movement of the sample and study it from all its aspects (8).

Preparation of vegetative organs epidermis

Leaf Epidermis

Soak the cuticles in distilled water for 1-2 minutes, depending on the plant species and the thickness of the leaf. After that, some modifications were made to the leaf cuticles, as follows (9): A fully grown leaf was taken from the middle of the plant, and the middle third was cut, including the middle vein and part of the blade. The upper and lower epidermis were obtained using the scraping method with high precision using two fine needles (insulin injection needles) and with extreme caution to avoid damaging the sample. The epidermis was removed using a sharp blade and transferred to a new glass slide using fine-ended forceps, and when a few drops of a commercial minor solution were placed on it, it was left in it for a period ranging between 1-2 minutes. Remove the minor solution by pouring it out by placing the glass slide at an angle, then transferring the prepared skin to another clean slide and putting drops of distilled water on it to eliminate the minor solution, with extreme caution to prevent the prepared skin from slipping. The prepared skin was then dyed by placing drops of safranin and glycerin dye on it, prepared in a 1:1 ratio, and left for 2-4 min. The slide cover was gently placed, and its edges were closed using transparent nail dye. The slides were marked with marking paper as they were ready for examination and study using the microscope.

Results

Adaxial epidermis cells

The results showed that the normal epidermal cells had overlaps, dimensions, and numbers between the upper and lower leaf surfaces of all the studied species and varieties in a single microscopic field. The upper epidermal cells were large in dimensions. They had regular geometric shapes ranging from quadrilateral and pentagonal to polygonal shapes with straight, curved and thick walls, as shown in Fig. 1.

The upper epidermal cells showed clear differences in their dimensions between the studied species and varieties, as the most significant average length of the normal epidermal

Table 1. Names of the genus, varieties, place and date of collection and family

Sample collection date	Family	Sample collection location	Genus	Species and varieties
April 16, 2023	Rhamnaceae Juss.	Baghdad / Al-Aziziya	*Ziziphus* Mell.	*Z.jujuba* Mill
March 28, 2023		Al-Haritha district		*Z.spina-christi*
November 8, 2023		Maysan/Al-Tayeb Governorate		*Z. nummularia*(Burm.f.) Weight & Arn
March 28, 2023		The second island		Var. Armouti
March 28, 2023		The second island		Var. Zaytony
March 28, 2023		Al-Haritha district		Var. Tofahy
March 28, 2023		The second island		Var. Armouti without
March 28, 2023		Abu Al-Khasib		Var. Armouti Tailandy
March 28, 2023		Abu Al-Khasib		Var. Tofahy Presidential
March 28, 2023		Abu Al-Khasib		Var. Tailandy Tofahy

cells was represented by the Tofahy variety belonging to the *Z. mauritiana* species, which reached up to 77.5 µm, as shown in Table 2 & Fig. 1. In comparison, the largest average width of the normal epidermal cells was 74.5 µm in the Armouti Tailandy variety belonging to the *Z. mauritiana* species. It was also found that the upper epidermal cell walls of the *Z. mauritiana* species were thicker than the rest of the species and varieties, especially the cells of the Armouti Tailandy species, Table 2 & Fig. 1, except for the Armouti without seed species, in which the epidermal cell walls were thinner. As for the nature of the upper epidermal cell walls, all species and varieties were straight, except for the *Z. jujuba* species, which was curved, an essential taxonomic characteristic, as shown in Table 2 & Fig. 1.

Stomata

The current study showed that all the studied species and varieties contain two types of stomata: anomocytic and hemiparacytic. The first type is characterized by the absence of subsidiary cells surrounding the stomata, while the second type contains only one subsidiary cell. However, the distribution of these stomata varied between the upper and lower epidermal surfaces of the studied species and varieties. This variation had a good classification value that enabled us to divide the species and varieties into one group, including those in which stomata are distributed on the upper and lower epidermal surfaces. However, the lower epidermal surface has more stomata, as is clear in most species of the species Z. *mauritiana* and the species *Z. nummularia* (Table 3).

The values of the stomatal index varied between the upper and lower epidermis, as the values of the stomatal index of the lower epidermis were greater than those of the upper epidermis in all the species and varieties studied. The average stomatal index of the lower epidermis in the species *Z. spina-christi,* which represents the highest value among the other species and varieties, is 23.25. For the upper epidermis, at the species level, the species *Z. spina-christi* and *Z.nummularia* had similar values, representing the highest values of 22.16 . As for the species level of the same species, the highest value of the stomatal index was in the epidermis of the variety *Z.mauritiana* cv. Tofahy presidential, and its value was 18.5, and the lowest value (3.26) was in the epidermis of the variety *Z.mauritiana* cv. Tofahy, as shown in Table 3.

Table 2. Surface appearance characteristics of the leaf epidermis in the studied species and varieties of the genus *Zizyphus* were measured with a micrometre

Nature of the walls	Upper epidermis		Species
Epidermal cells Ordinary	Width of Ordinary epidermal cells	Length of Ordinary epidermal cells	
Straight	(87.5–57.5) 68.5	(87.5–52.5) 62.75	*Z. mauritiana* cv. Armouti
Straight	(85.5–57.5) 65.5	(87.5-57.5) 73.5	*Z. mauritiana* cv. Zaytony
Straight	(80 .0– 55.0) 66.75	(155 .0-57.5) 77.5	*Z. mauritiana* cv. Tofahy
Straight	(75.0–50.0) 57.65	50.0(62.5-55.25)	*Z. mauritiana* cv. Armouti without nucleus
Straight	(85.0–57.5) 74.5	(75.0–57.5) 67.5	*Z. mauritiana* cv. Armouti Tailandy
Straight	(80 .0– 50 .0) 62.5	(87.5–62.5) 73.5	*Z. mauritiana* cv. Tofahy Presidential
Straight	(100.0–52.5) 65.75	(90. 0– 55.0) 74.8	*Z. mauritiana* cv. Tofahy Tailandy
Straight	(75.0–52.5) 57.67	(80.0–50 .0) 60.75	*Z. spina–christi*
Straight	(87.5–57.5) 68.3	(87.5–50 .0) 67.75	*Z. nummularia*
Curved	(82.5–37.5) 62.25	(87.5–50.0) 68.5	*Z. jujuba*

Table 3. Characteristics of the stomata in the leaf epidermis of the studied species and varieties of the genus *Zizyphus*, measured with a micrometre

Number of stomata per 1 mm²	Adaxial epidermis cells		Species
	Width of the stoma	Length of the stoma	
(70.0–51.0) 60.9	(87.5–62.5) 69.75	(75.0–55.0) 65.0	*Z.mauritiana* cv. Armouti
(69.5–51.5) 61.3	(76.5–61.5) 68.9	(75.6–54.5) 64.9	*Z.mauritiana* cv. Zaytony
(72.0–177.0) 120.7	(90.0–57.5) 67.5	(77.5–47.5) 63.5	*Z.mauritiana* cv. Tofahy
(20.0–41.0) 36.4	(67.5–57.5) 65.0	(67.5–50.0) 50.0	*Z. mauritiana* cv. Armouti without nucleus
(35.0–144.0) 89	(75.0–62.5) 71.25	(82.5–62.5) 70.3	*Z. mauritiana* cv. Armouti Tailandy
(73.0–145.0) 102	(75.0–57.5) 63.75	(87.5–37.0) 55.25	*Z. mauritiana* cv. Tofahy Presidential
(70.0–179.0) 110	(60.0–57.5) 61	(65.0–50.0) 58.5	*Z. mauritiana* cv. Tofahy Tailandy
(51.0–70.0) 61.3	(60.0–45.0) 53.0	67.5–27.5) 44.75	*Z. Spina–Christi*
(108.0–259.0) 172.9	(85.5–57.5) 74.8	(87.5–57.5) 62.9	*Z. nummularia*
(49.0–11.0) 35	(62.5–52.5) 59.25	(37.0–57.5) 60.75	*Z. Jujuba*

Fig. 1. Va

The shapes of the stomata varied between elliptical, semi-circular, or circular in all species and varieties. Still, the dimensions of the stomata varied between the upper and lower epidermis within the same species or variety, as the dimensions of the stomata on the surface of the upper epidermis were more significant than the dimensions of the stomata on the surface of the lower epidermis, In general, the dimensions of the stomata on the surface of the upper epidermis were more significant than their dimensions on the lower epidermis. The highest average length of the stomata on the upper epidermis was in the *Z. mauritiana* cv. Armouti Tailandy variety was 70.3 µm, while the lowest average was 43.75 µm in *Z. jujube*. The average width was 74.8 µm in the *Z. nummularia* variety, and the lowest average was 53.0 µm in the *Z. spina-christi* variety (Table 3).

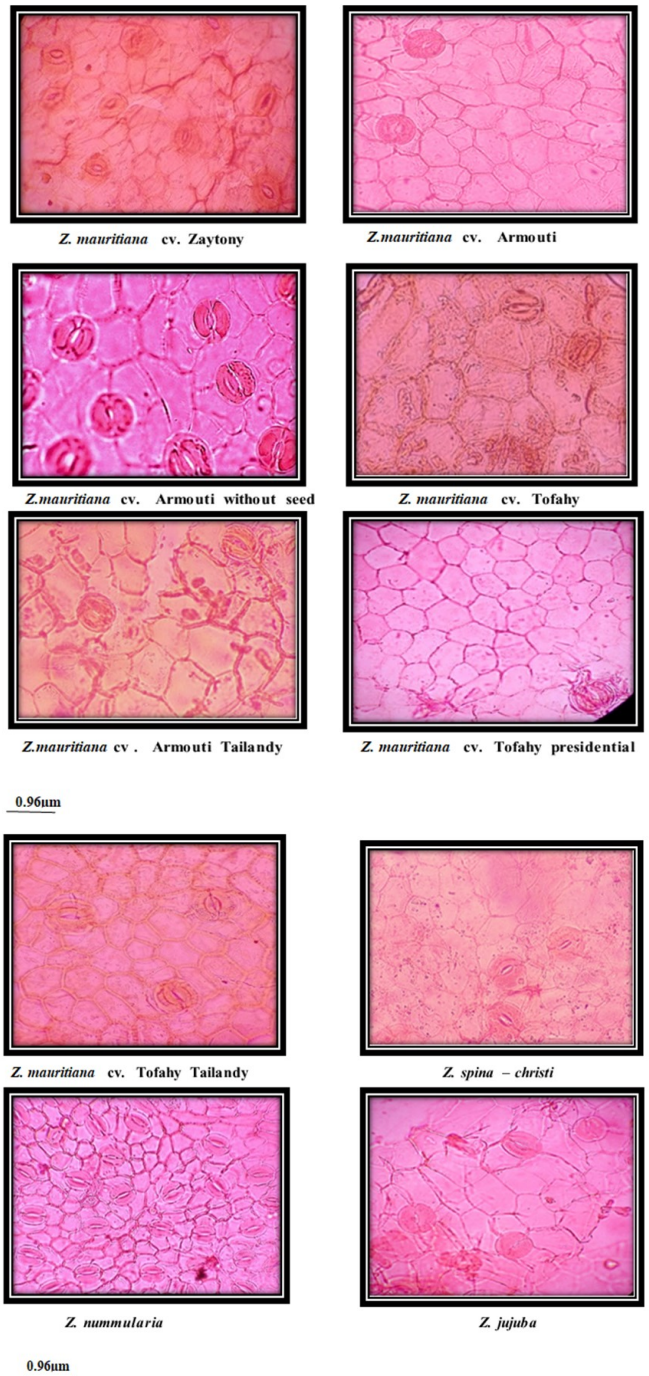

Fig. 1. Variations like the normal cell walls in the upper epidermis of the species' leaves and varieties of the genus *Zizyphus* under study strength (40 x).

Discussion

The current study showed many anatomical differences, mainly between species and sometimes between taxa, which could contribute to supporting the anatomical value of phenotypic traits.

Adaxial epidermis cells

The upper and lower epidermal cells of the studied species and varieties showed apparent differences in their dimensions. Although *Z. mauritiana* and *Z. spina-christi* were found in the same environmental conditions, the dimensions of the upper epidermal cells of the former were more significant than those of the latter, which made it a distinctive feature of this species. *Z. nummularia,* the upper epidermis of the varieties of this species, was significant in dimensions, unlike *Z. nummularia*, in which the upper epidermal cells were large in dimensions. These facts reflected what was found (10).

This change in the results indicates that the difference in the dimensions of the epidermal cells is not only due to the change in the environmental conditions surrounding the plant but may be attributed to the nature of the genetic characteristics specific to the species. The difference was also evident in the thickness of the surface walls of the upper epidermal cells, as they were thicker in most varieties of the *Z. mauritiana* species than in the rest of the species, especially the cells of the Armouti Tailandy species, even denser than the walls of the upper epidermal cells of the *Z. nummularia* species, despite the harsh conditions experienced by this species. This variation in the thickness of the cell walls may be due to the nature of the environmental conditions surrounding the plant, or it may be due to the nature of the genetic characteristics specific to that species. The presence of cells containing mucilage in the upper epidermis of leaves and external cavities containing these substances in the veins of the leaf and the cortex of the stem in the species *Z. mauritiana* is consistent with our present study's results (11).

Stomata

The variation in the distribution of stomata on the upper epidermis was evident among species and sometimes among varieties of the same species. This variation helped in including species and varieties in one group, as mentioned in the results. This reflects the close evolutionary relationship between them. Then, the frequency of stomata on the upper epidermis *of Z. nummularia* is higher than that of the rest of the species. This may seem natural for *Z. nummularia* due to the nature of the desert environment. The increase in stomata frequency may be primarily associated with dry or semi-dry environmental conditions. The lack of stomata on the upper epidermis of *Z. Jujuba* was a distinctive feature (12).

The stomata were spread on both sides of the leaf surfaces of the studied species and varieties and between the anomalous and semi-parallel types. This differs from what was recorded earlier in, which spread on the lower epidermis only and anomalous type, except for the species *Z. jujuba*, which includes the anicocytic type, which was not shown in the results of our study (12). The variation in the dimensions of the stomata in this species may be the reason for this scarcity. In contrast, the variation in the dimensions of the stomata is different, some small and some large, in the species of *Z. mauritiana* and this may explain their increased numbers on the surface of the upper epidermis.

Conclusion

The variation in the stomata frequency on the upper epidermis surface between species was insignificant. However, the frequency of stomata on the surface of the upper epidermis of *Z. mauritiana* species was more significant than its frequency in the rest of the species, followed by *Z. nummularia*, then *Z. jujuba*, then *Z. spina* - christi. The low frequency of stomata reflected the cold and temperate nature of the plant in which the plant was found.

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None

Authors' contributions

All authors contributed equally to this research.

Compliance with ethical standards

Conflict of interest: Authors have no conflict of interest

Ethical issues: None

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