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Vegetative Anatomy as a Source of Taxonomic Characters in Rosaceae in Iraq

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ASPECTS of variations in the stem, leaf, and petiole anatomy of *Crataegus monogyna* Jacq., *Malus domestica* Borkh., *Prunus armeniaca* L., *Prunus domestica* L., *Prunus avium* (L.) L., *Prunus persica* (L.) Batsch, *Prunus spinosa* L., *Pyrus communis* L., and *Rosa* × *damascena* Herrm. (Rosaceae) have been recorded compared to fresh specimens. The present study disclosed that the anatomical characteristics of the leaf and stem are important in differentiating different species, such as leaf thickness, mesophyll, vascular bundle, and the upper and lower epidermis of the leaf. The only vascular bundle of the leaf mid vein is consistently kidney-shaped except in *P. spinosa* where it is circular. The petiole outline in the cross section, was between kidney and circular in all species. The data matrix was analyzed numerically using the PAST program. Species were divided into two groups based on cluster analysis and similarity percentage as species belonging to *Prunus* formed one group and *R. damascena* and *P. persica* were placed in another group. The current study indicates the taxonomic usefulness of the largely untapped source of anatomical attributes of Rosaceae.

Keywords: Anatomy, *Crataegus*, *Malus*, PAST, *Prunus*, *Pyrus*.

Introduction

Rosaceae is economically deemed important because most fruit trees and shrubs, namely apples, apricots, peaches, and strawberries belong to this family. These are herbaceous or woody plants, often perennial and sometimes annual, evergreen or deciduous trees, shrubs or semi-shrubs, alternating leaves simple or compound pinnate, pentamerous dioecious flowers, sepals, and petals free or united with the receptacle, and fleshy or dry fruit. Rosaceae comprises 140 genera and 3500 species spread all over the world (Al-Mayah et al., 2016).

It is worth mentioning that anatomical characters have remarkable importance and taxonomic value not less than phenotypic characters and other types of classification if anatomy is used to diagnose and classify plants and determine genetic relationships and the extent of similarities and differences between taxonomic ranks of family, genus, species, and other levels (Al-Kateb, 1988).

Some anatomical studies elaborated on diverse groups of Rosaceae, including research by Zamani et al. (2008) shedding light on ten *Pyrus* species belonging to the four sections *Pashia, Pyrus, Xeropyrenia, Argyromalon,* and one specimen farmed close to *P. syriaca.* The ten species are *Pyrus boissieriana* Buhse, *P. turcomanica* Maleev, *P. kandevanica* Ghahreman and Khatamsaz, *P. hyrcana* Fedor, *P. syriaca* Boiss., *P. elaeagnifolia* Pall, *P. glabra* Boiss., *P. oxyprion* Woron., *P. salicifolia* Pall., and *P. mazanderanica* Schönbeck-Temesy. Among 43 studied anatomical characters, 29 differed in

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miscellaneous locations, seven were similar in all species, and four had a taxonomic value and could be used to distinguish the species. These characteristics entail the multiplicity of macular cell layers in the parenchyma tissue and its density in the upper epidermis, compared to the lower epidermis, which was characterized by the lack of pillar cells in the parenchyma tissue.

A study conducted by Faghir et al. (2011) showed the importance of leaf anatomical characters in the taxonomy of 27 species of *Potentilla* (represented by sections), *Tylosperma* Botsch., *Schistophyllidium* (Juz. ex Fed.) Ikonn., *Drymocallis* Fourr. ex Rydb., and *Sibbaldia* L. available in Iran. They studied the locations of stomata on the leaf surface, types of mesophyll, crystal locations, as well as the shape and patterns of vascular bundles. Song & Hong (2018) focused on the anatomical characters of 16 species belonging to the *Sorbarieae* sub-group of Rosaceae, including the genera *Adenostoma* (two spp.), *Chamaebatiaria* (one spp.), *Sorbaria* (six spp.), *Spiraeanthus* (two spp.), and *Lyonothamnus* (one spp. and four varieties). The researchers stated that anatomical characters are significantly important in separating the species and genera in this sub-group.

In Iraq, there were several studies on the genera or species of this family. Al-Rfaish (2012) performed a comparative study on the anatomical characters of the species of some genera of the Rosaceae-Pomoideae in Iraq. They examined the epidermis characters of the leaves and their petiole sections. Similarly, Dalalbashi (2019) used the anatomical characters of the stems of five cultivars of Italian pears grown in northern Iraq, namely *Abate fetel, Conference, Beurre hardy, Bonne louise,* and *Doyenne comice*. This study demonstrated variations in some anatomical characters, such as epidermis thickness, stem collenchyma and parenchyma, xylem shape, cortex thickness, and the number of vascular bundles. Such characters were of great importance in isolating and diagnosing these species. Al-Taie et al. (2020) studied the vegetative, reproductive, and anatomical characters of some species of the genus *Malus*. Their results revealed that the leaves of the studied species had very diverse patterns of venation in all species is reticulate and pinnate form, where all the secondary veins and their branches ended at the leaf edge.

Materials and Methods

Fresh samples of *C. monogyna*, *Malus domestica* Borkh., *Prunus armeniaca* L., *Prunus domestica* L., *Prunus avium* (L.) L., *Prunus persica* (L.) Batsch, *Prunus spinosa* L., *Pyrus communis* L. and *Rosa* × *damascena* Herrm. were collected from distinct Iraq governorates, including the mountains of Kurdistan. The specimen of *C. monogyna* was identified using the flora of Iraq (Guest, 1966). All other taxa are well-known cultivated trees, and their identity needed no further confirmation. Names of taxa and their author citations were verified using the website The Plant List (The plantlist.org).

Stem, leaf blade, and petiole samples of all species were fixed in formalin-acetic acid-70% ethyl alcohol (FAA) mixed in ratios of 5:5:90 ml for 48h and were kept in 70% ethyl alcohol until embedding in paraffin wax and sectioning according to the procedure described by Johansen (1940). Sections were cut using a rotary microtome, stained in safranin (1% aq. solution) and fast green (1% in abs. ethanol), mounted in Canada Balsam, and photographed using a light microscope with a digital camera (type DCE-2). Measurements of tissues were made using a calibrated eyepiece micrometer. The PAST program (Hammer, 2018) was used for the dendritic diagram of some anatomical characteristics of the studied species as 28 anatomical characteristics were selected.

Results and Discussion

The anatomical characters of the stem are among the important ones in plant anatomy and are used to identify the species and genera based on the stem thickness, vascular bundle, cortex, epidermis, xylem, phloem, cortical bundle, pith, and the types and shapes of crystals (Table 1 and Fig. 1). Figure 1 shows the variations in the average epidermis thickness of the stem. We observed that the highest value was 25.8µm for *M. domestica*, followed by 24µm for *P. domestica*. On the other hand, the lowest epidermal thickness was 17.3 µm in *P. avium*. As for the rest of the species, the values were close and had a range of 17.5-19.4. Cells of most species were distinguished by their circular or semi-circular shape (Plate 1).

The thickness of chlorenchyma was larger than

TABLE 1. Anatomical characters of the stem in nine species of the Rosaceae family; all measurements are in µm (Ch: Chlorenchyma; Par: Parenchyma; 0 = Absent)

Species	Stem thickness	Vessel thickness	Cortex thickness		Epidermis thickness	Xylem	Phloem	Pith
			Ch	Par				
C. monogyna	1921 (1500-2500)	330 (275-375)	30 (20-40)	52 (40-60)	17.6 (16-20)	210 (175-250)	140 (125-175)	550 (500-600)
M. domestica	3635 (3425-3875)	556 (500-625)	64 (50-80)	304 (220-400)	25.8 (20-30)	281.6 (250-300)	180 (150-220)	1350 (1250-1500)
P. armeniaca	1587 (1450-1750)	345 (300-400)	40 (30-50)	50 (40-60)	17.6 (15-20)	170 (150-200)	156.5 (125(175)	625 (550-700)
P. domestica	1980 (1872-2075)	530 (500-575)	0	170 (150-200)	24 (20-30)	260 (220-300)	116 (100-140)	425 (375-450)
P. avium	1785 (1675-2025)	270 (240-350)	42 (35-50)	55 (45-65)	17.3 (16.5-19)	165 (125-185)	157 (135-160)	445 (390-540)
P. persica	1625 (1430-1850)	240 (185-275)	0	116 (102-128)	17.5 (15-21)	185 (165-205)	142 (130-157)	590 (540-650)
P. spinosa	2785 (3250-3500)	1260 (1200-1300)	110 (75-125)	215 (175-250)	19.2 (18-21)	550 (500-625)	480 (425-500)	490 (375-550)
P. communis	1350 (1225-1500)	255 (245-280)	0	48 (35-60)	18 (17-22)	178 (150-210)	155 (135-190)	520 (430-580)
R. damascena	870 (850-900)	166 (150-180)	58 (50-70)	44 (40-50)	19.4 (18-20)	72 (60-80)	70 (60-80)	476 (450-500)

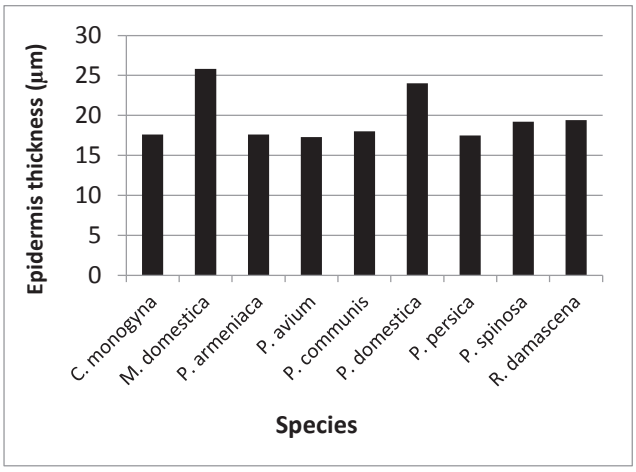

Fig. 1. Average thickness of the epidermis of the stem in the studied species

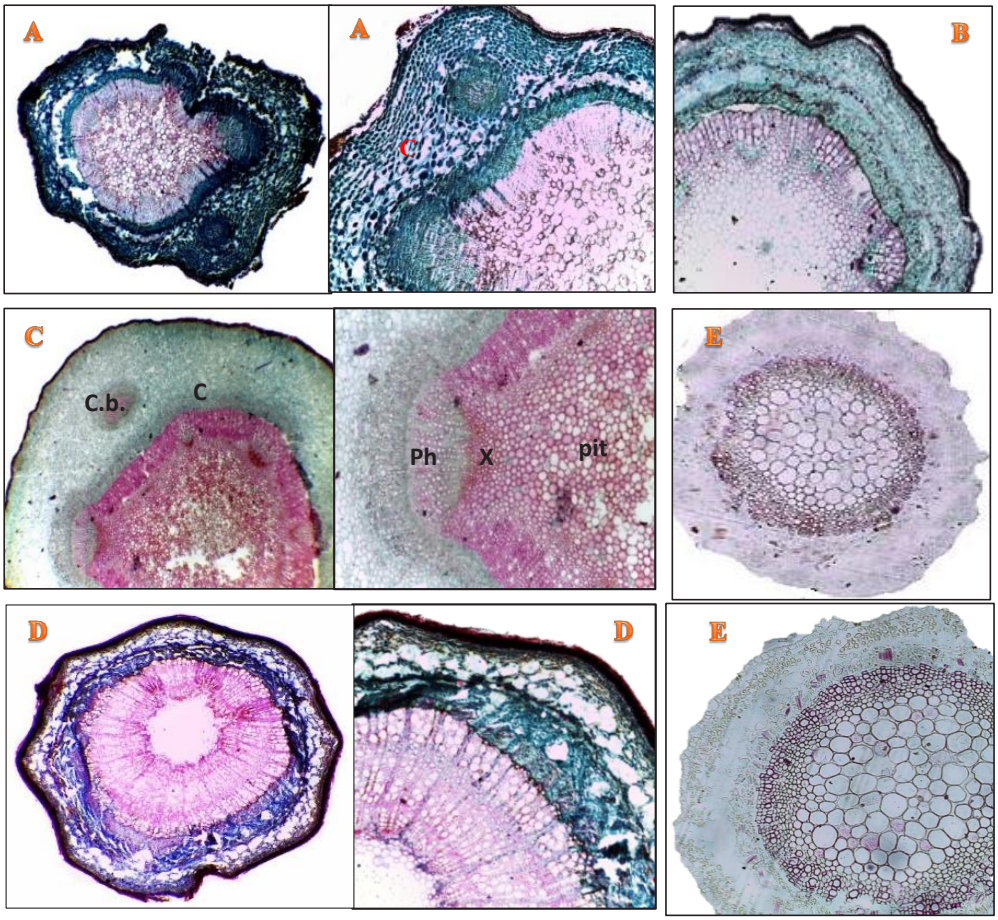

Plate 1. Cross sections in the stems of five species of the Rosaceae family, (A) *C. monogyna*, (B) *P. armeniaca*, (C) *M. domestica*, (D) *P. domestica*, (E) *R. damascena* [C: Cortex; Cb: Cortical bundle; Ph: Phloem; pit: Pith; X: Xylem]

parenchyma, as the highest chlorenchyma thickness of 110µm was recorded in the stem cortex of *P. spinosa*, while the lowest was 30µm in *C. monogyna*. In the three species *P. communis*, *P. persica*, and *P. domestica*, the cortex was free of chlorenchyma. Concerning the parenchymal tissue, the highest thickness was 304µm in *M. domestica*, and the lowest was 44µm in *R. damascena* (Figs. 2 and 3). The cortical bundle was observed in all assessed species except *C. monogyna* and *M. domestica*. The latter species contained cortical bundles within the tissues of the stem. It was noticed that the first species contained two layers of the bundle and the second species contained 2-4 layers.

Furthermore, Fig. 3 demonstrates variations in the stem thickness for the studied species of the Rosaceae. The thickest samples of the stem were *M. domestica*, which averaged about 3635µm, followed by *P. spinosa* with stems reaching 2785µm in thickness. The lowest average thickness was 870µm in *R. damascena.* The average thickness of the stem in the rest of the studied species was 1350-1980µm. This trait is considered important in separating the studied samples. This result was consistent with a study conducted by Al-Mousawi et al. (2019), which indicated that stem characteristics, such as size, shape, and cortical layers number, are tremendously valuable in separating species.

Figure 4 pinpoints the variations in the thickness of the vascular bundle of the stem. It was found that the highest and lowest thickness of the vascular bundle was 1260 and 166µm in *P. spinosa* and *R. damascene,* respectively. The same Table indicates that other species had values in the range of 240-530µm. The thickness of xylem and phloem differed between the studied species as the thickest xylem cross section was 550µm for *P. spinosa*, and the thinnest was 72µm for *R. damascena*. Moreover, the highest rate of phloem tissue was for *P. spinosa*, which measured 480µm, and the lowest of 70µm was recorded for *R. damascena* (Fig. 5). In addition, variations in the thickness of the pith were observed

in the studied species. The highest pith thickness was 1350µm in *M. domestica*, while the lowest was 425µm in *P. domestica* (Fig. 6). Furthermore, there are two types of crystals in the evaluated species, namely druses and prismatics. Druses are found in all species, except *R. damascena* which contains only the prismatic type. *M. domestica* and *P. domestica* contain both types. The density of the two types in most species was few to medium, except for *M. domestica*, *P. domestica,* and *P. spinosa*, where crystals are abundant (Table 1 and Plate 3).

In addition, Table 2 and Plate 2 show the variations in the leaf cross sections of the studied species of the Rosaceae. Such variations are important in separating the studied species, namely the thickness of the leaf, columnar and spongy layer of the mesophyll, the thickness of the middle vein and its vascular bundle, and the thickness of the upper and lower epidermis. It was found that the highest leaf thickness was 202.5µm for the species *M. domestica*, and the lowest was 85µm for the species *P. domestica*. The shape of epidermal cells varied from round to oval, with some being rectangular (Fig. 7 and Plate 2).

Focusing on the leaf mesophyll which consists of two layers of columnar (or palisade) and spongy cells, it was disclosed that the thickness of the two layers differs between species, and the columnar layer is thicker in species *P. persica* and *M. domestica*, with the average thickness of 108 and 102µm, respectively. The lowest thickness of 37µm was found in *P. armeniaca*. The species *M. domestica*, *P. persica,* and *P. communis* were characterized by recording the highest average thickness of the spongy layer of 72, 72, and 78µm, respectively, whereas the lowest was 35 µm in *P. avium*. Regarding the shape, the cells were elongated in the columnar layer and lobed in the spongy, which is consistent with the results of Al-Rfaish (2012) (Table 2, Fig. 8, and Plate 2).

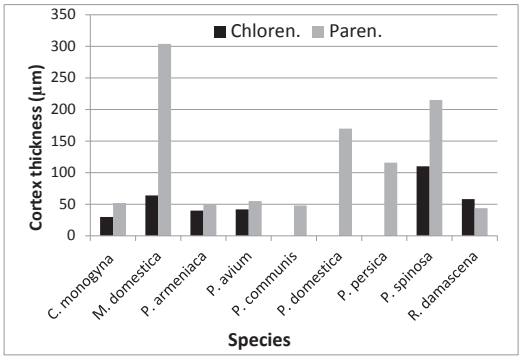

Fig. 2. Average thickness of the stem cortex in the studied species. Chloren. = chlorenchyma; Paren. = parenchyma.

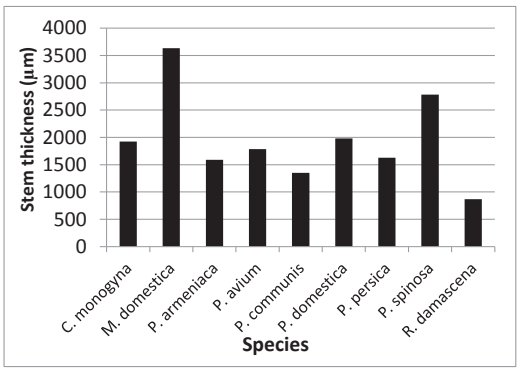

Fig. 3. Average stem thickness in the studied species.

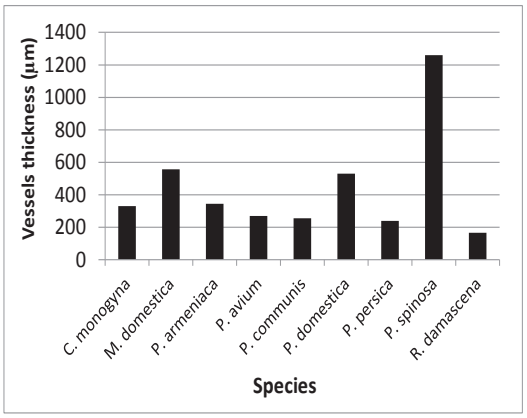

Fig. 4. Average thickness of the vascular bundle of the stem in the studied species.

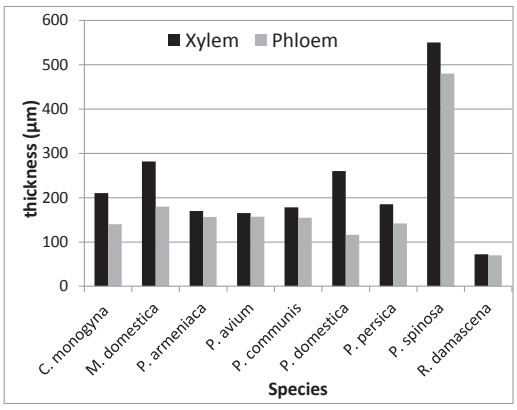

Fig. 5. Average thickness of the xylem tissue and the phloem of the stem in the studied species.

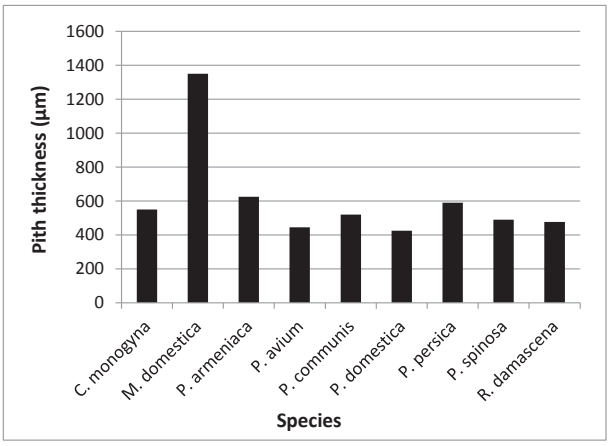

Fig. 6. Average thickness of the pith of the stem in the studied species

TABLE 2. Anatomical characters of the leaves of nine species of Rosaceae; all measurements in µm

Species	Leaf thickness	Mesophyll thickness		Mid veins	Vessels in Mid vein	Epidermis thickness	
		Palisade layer	Spongy layer			Upper	Lower
C. monogyna	166 (140-200)	52 (40-60)	62 (50-70)	208 (200-220)	96 (80-110)	11.8 (10-14)	6.6 (5-8)
M. domestica	202.5 (180-220)	102 (90-110)	78 (70-90)	1000 (875-1125)	390 (350-450)	16 (12-20)	8.4 (7-10)
P. armeniaca	200 (180-220)	42 (35-50)	55 (50-60)	425 (400-450)	200 (180-220)	37 (30-40)	15.2 (12-19)
P. domestica	85 (75-100)	37 (30-40)	44 (40-50)	280 (250-300)	150 (140-160)	12.4 (10-15)	6.8 (5-10)
P. avium	138 (130-150)	50 (40-60)	35 (30-40)	604 (550-650)	212 (190-250)	18 (15-20)	9.8 (8-12)
P. persica	206 (200-220)	108 (100-120)	72 (60-80)	1026 (980-1110)	434 (400-480)	14.8 (14-16)	9.6 (8-11)
P. spinosa	146 (130-160)	48 (40-55)	51 (40-60)	470 (450-500)	238 (200-270)	14.4 (12-18)	11.6 (10-13)
P. communis	151 (130-170)	44 (30-60)	72 (60-80)	308 (280-350)	158 (150-170)	12 (10-14)	8.2 (6-10)
R. damascena	134 (120-150)	68 (60-80)	46 (40-50)	520 (500-550)	228 (200-250)	2.8 (10-15)	7.6 (6-10)

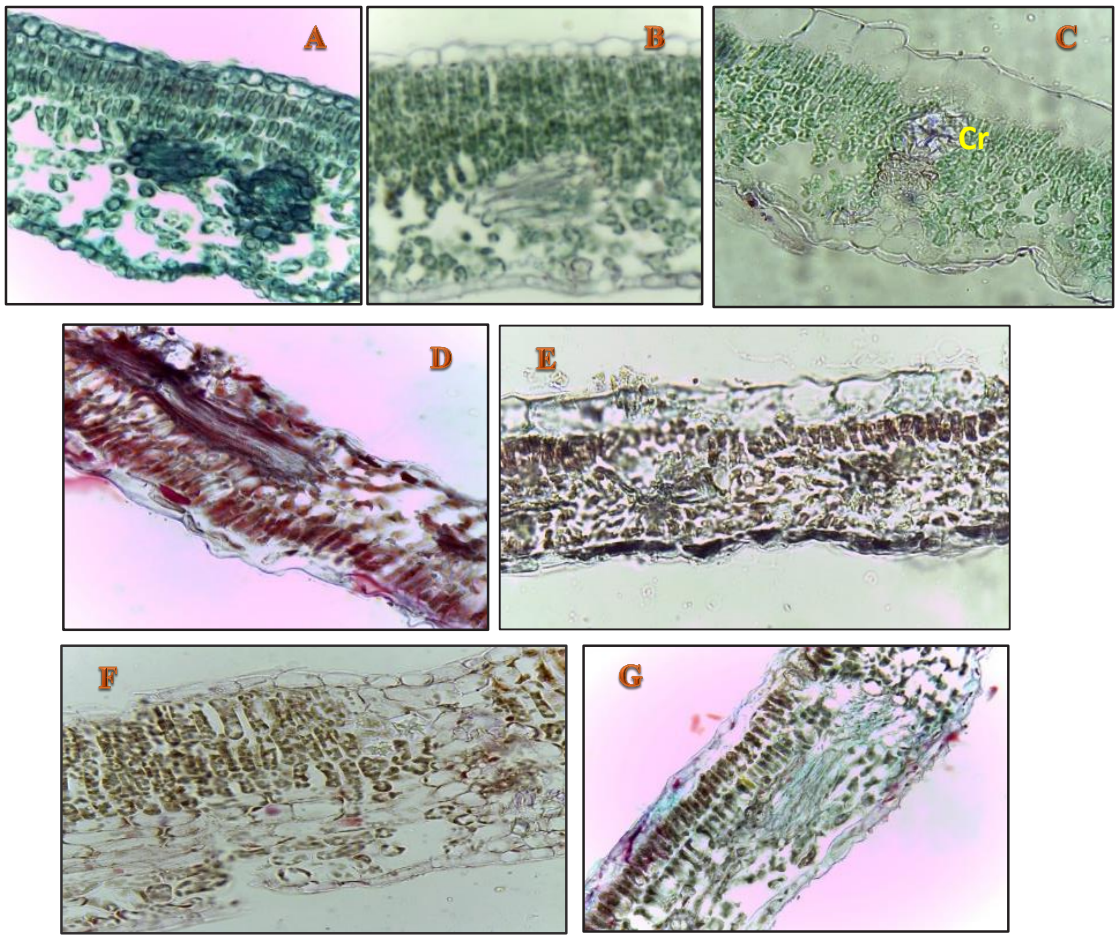

Plate 2. Photomicrographs of transverse sections in the leaf blades of seven species of Rosaceae, (A) *C. monogyna;* (B) *M. domestica;* (C) *Prunus armeniaca;* (D) *P. avium;* (E) *P. domestica;* (F) *P. spinosa;* (G) *Pyrus communis* [Cr: Crystal]

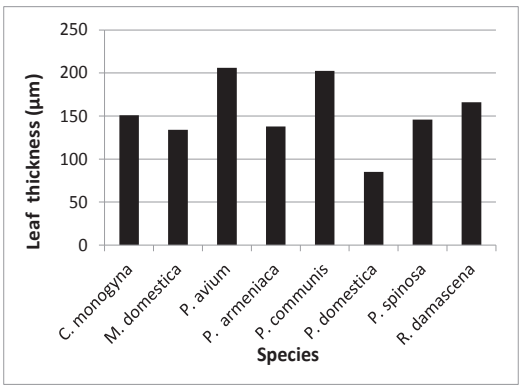

Fig. 7. Average leaf thickness in the studied species

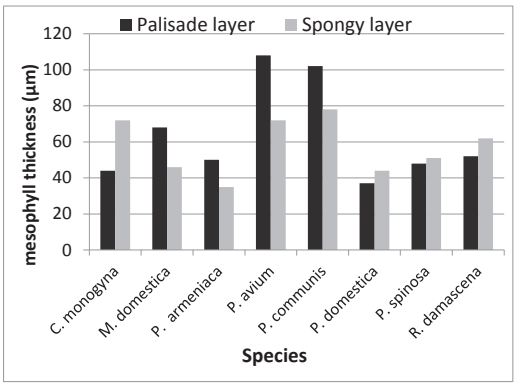

Fig. 8. Average thickness of the mesophyll layer in the studied species

P. persica had the highest average thickness of the mid vein (1026µm) and vascular bundle (434µm), followed by *M. domestica*, which was 1000µm (Fig. 9). The lowest average thickness of the mid vein and vascular bundle was 208 and 96µm in *C. monogyna*, respectively. The intensity of the enlarged part of the mid vein differed from the lower side. It was very prominent in the species *P. persica*, *M. domestica,* and *P. domestica*, while it was medium in *P. armeniaca*, and low in *C.*

monogyna. The upper side of the middle vein was severely concave in *C. monogyna,* concave in *P. armeniaca*, and anticline in *P. persica* and *M. domestica* (Table 2 and Plate 3).

According to Table 2, Plate 2 and Fig. 10, the upper and lower epidermal cells had variable thicknesses. The highest upper epidermal thickness was 37µm in *P. armeniaca*. The same species also had the highest average thickness of the lower epidermis (15.2µm), and the lowest thickness of the upper epidermis was 11.8µm in *C. monogyna*.

Moreover, the number of palisade cell layers varied from one species to another. It was 1-2 rows in *P. armeniaca* and *P. communis*, and 2-3 in *C. monogyna*, *M. domestica,* and *P. armeniaca*. Leaves of *P. persica* are distinguished by having 3-4 rows of columnar cells. All species only have one vascular bundle in the leaf middle vein, and all types of bundles were kidney-shaped except in *P. communis*, which was circular (Plate 3). Many crystals were observed in the tissues of the leaves. The shape and density of crystals are different in distinct species. It was found that most species contained Rosette type (or druses) crystals with a density of little to medium, except for the two species *P. spinosa* and *R. damascena*, in which crystals were abundant. The two species *M. domestica* and *P. persica* had two types of crystals, namely druses and prismatic types. There were few prismatic ones, and *C. monogyna* did not have any type of crystals.

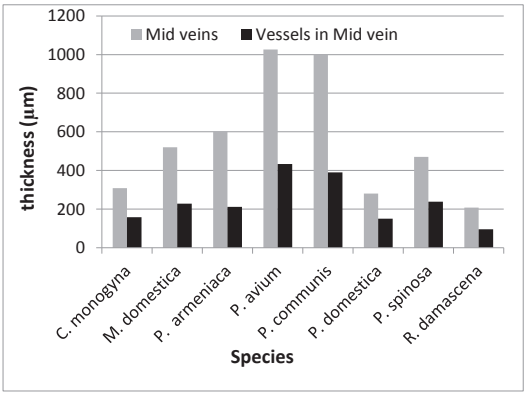

Fig. 9. The average thickness of the mid vein and its bundle in the leaf in the studied species

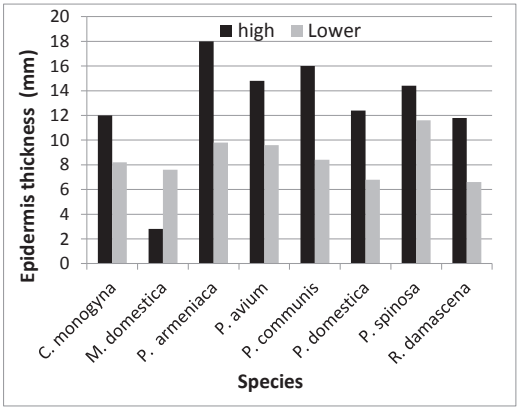

Fig. 10. Average thickness of the upper and lower epidermis of the leaf in the studied species

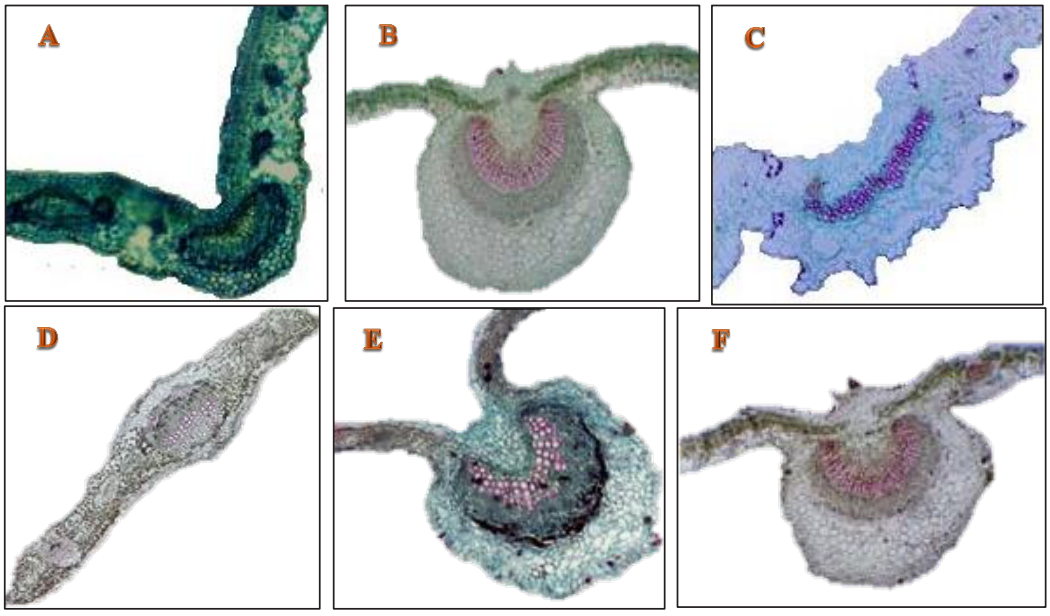

Plate 3. Photomicrographs of cross sections illustrating the mid vein anatomy of six species of Rosaceae, (A) ***C. monogyna;*** **(B)** ***M. domestica;*** **(C)** ***P. armeniaca;*** **(D)** ***P. communis;*** **(E)** ***P. domestica;*** **(F)** ***P. spinosa***

Results of Plate 4 unveiled that the leaf petiole had considerable taxonomic importance, and could be used for differentiating some studied species from each other, especially in terms of petiole shape, vascular bundles, and the number of side bundles (Table 3). The general petiole shape differed remarkably between the species. It was kidney-shaped in *C. monogyna*, *P. domestica,* and *P. persica*, arc-shaped with side appendages in *R. damascena* and *P. spinosa*, and strongly arc-shaped in *P. communis*. In *P. avium,* the petiole outline in cross sections was strongly arc-shaped with two wings, while it was circular to semi-circular in *M. domestica* and circular in *P. armeniaca*.

The number of lateral bundles in the petiole varied from species to species, and there was only a single main bundle in the leaf petiole of all nine species. Lateral bundles are 2-4 in *P. avium*, *P. spinose,* and *R. damascene*, and 1-2 in *P. domestica*. The petioles of all other species had no lateral bundles. The average thickness of the main bundle of the petiole is similar in most species, with a range of 200-375µm, except for *P. spinosa*, which was recorded to have the lowest thickness of about 92.8µm.

Our findings showed that the petiole thickness was important in differentiating the studied species. Some species were very thick, such as *M. domestica*, in which the average thickness of the petiole was 1612µm. In terms of the two species *P. persica* and *P. armeniaca*, the average thickness was 1190 and 1185 µm, respectively. The lowest thickness of 248 µm was recorded for *P. spinosa*. The main bundle shape in the petiole was arced to strongly arced for all species. Concerning the presence of crystals and their shapes, two types of crystals were recorded, namely druses and prismatic. Most of the studied species had few to many of the first type crystals, except for *M. domestica*, which contained numerous crystals of both patterns. Considering *P. persica*, the crystals were prismatic and were present in a high density.

A cluster analysis of the studied species of Rosaceae relying on some studied anatomical characters (Diagram 1) demonstrated that the two species *P. spinosa* and *P. avium* were closely related, with a similarity rate of 70%. Likewise, the two species *R. damascena* and *P. persica* were correlated to one group with a similarity rate of 53.8%. To sum up, our findings revealed the formation of two main groups. The first group consisted of two branches, which is the connection of the two species *R. damascena* and *P. persica*, along with *M. domestica* was associated. The second group included the main group of *P. spinosa* and *P. avium*, and a subgroup involved the rest of the studied species.

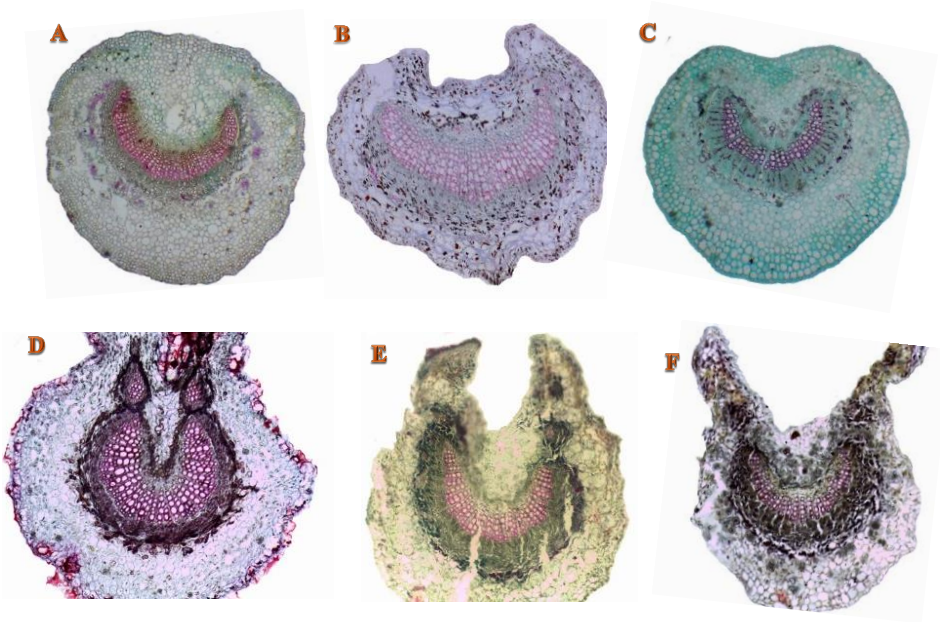

Plate 4. Photomicrographs of cross sections illustrating the petiole anatomy of six species of Rosaceae, (A) *M. domestica;* (B) *P. communis;* (C) *P. armeniaca;* (D) *P. avium;* (E) *P. spinosa;* (F) *R. damascena*

TABLE 3. Anatomical characters of petiole in nine species of Rosaceae; all measurements in µm

Species	Petiole shape	Thickness of lateral vascular bundle	Thickness of petiole	Vascular bundle shape
C. monogyna	Kidney-shaped	245 (180-275)	325 (370-450)	Strongly arc-shaped
M. domestica	Semi-circle, circle	335 (250-375)	1612 (1500-1675)	Arc-shaped
P. armeniaca	Semi-circle, circle	370 (350-400)	1185 (1125-1250)	Arc-shaped
P. domestica	Kidney-shaped	375 (325-450)	712.5 (625-775)	Arc-shaped
P. avium	Strongly arc-shaped with two wings	240 (200-275)	906.5 (800-1000)	Strongly arc-shaped
P. persica	Kidney-shaped	300 (250-375)	1190 (1125-1250)	Arc-shaped
P. spinosa	Arc-shaped with side appendages	92.8 (175-300)	248 (550-750)	Arc-shaped
P. communis	Strongly arc-shaped	372 (350-400)	630 (600-670)	Arc-shaped
R. damascena	Arc-shaped with side appendages	238 (220-250)	620 (600-650)	Arc-shaped

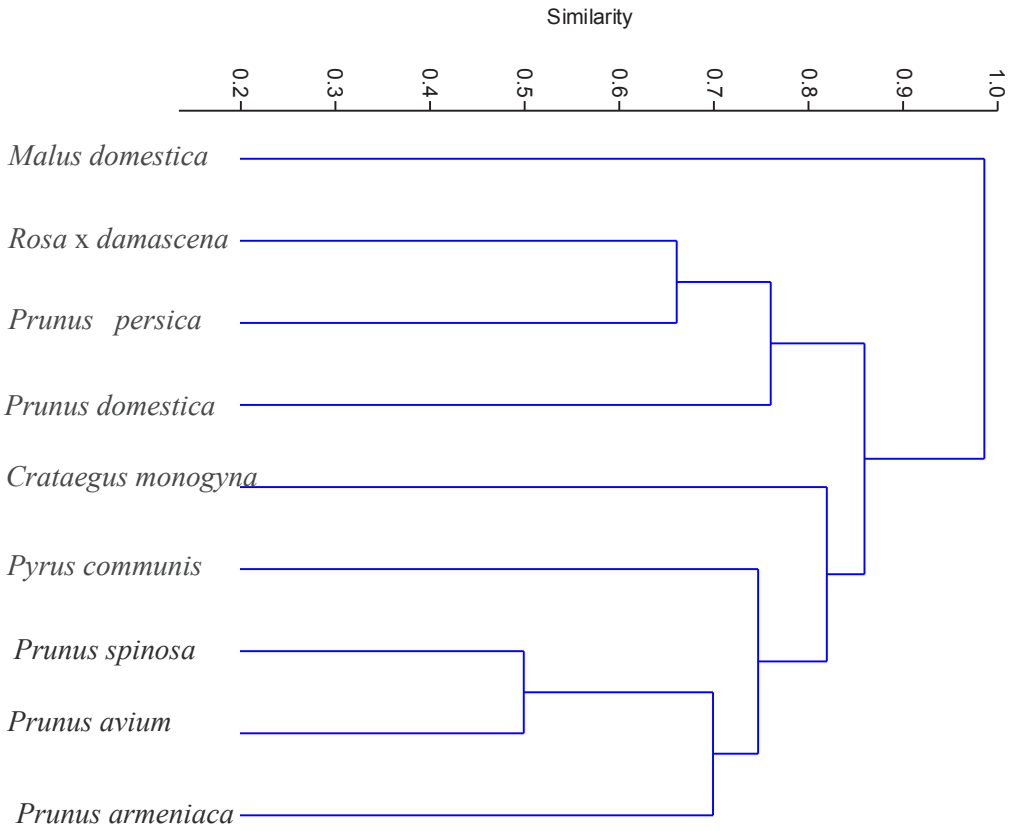

Diagram 1. Dendritic diagram showing the taxonomic relatedness of nine species of the Rosaceae family based on 28 characters of vegetative anatomy recorded comparatively and analyzed numerically under the program PAST

Conclusion

Anatomical features are important in the identification and separation of species in the family Rosaceae, such as the thickness of the stem, the upper and lower epidermis of the leaf, the number of bundles of the leaf stalk, and the presence or absence of crystals.

Competing interests: The authors report no conflicts of interest regarding this work.

Authors' contributions: EMA, and WMT proposed the idea of this study, designed the experimental work and made the measurements. WMT analyzed and interpreted the data and wrote the manuscript, participated with WME in the analysis and interpretation of the data, revised the manuscript, All authors participated in the drafting of the manuscript and have read and approved the final draft.

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التشريح الخضري كمصدر للخصائص التصنيفية في العائلة الوردية

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درست الخصائص التشريحية لتسعة أنواع تابعة لخمسة أجناس من العائلة الوردية وهم *Crataegus monogyna* Jacq. و *Malus domestica* Borkh و *Prunus armeniaca* L و *Prunus domestica* L و *Prunus avium* (L.) L و *Prunus persica* (L.) Batsch و *Prunus spinosa* L.، *Pyrus communis* L. and *Rosa* x *damascena* Herrm. تم تسجيل المعدلات لقياسات الأنسجة المدروسة (ساق والورقة) المعرضة للتغيير مع التغيرات في الظروف البيئية السائدة، وعمر النبات، وكشفت الدراسة أن خصائص المقاطع التشريحية للورقة والساق مهمة في فصل الأنواع، مثل سمكة الورقة، والنسيج المتوسط والعرق الوسطي، الحزمة الوعائية، والبشرة العليا والسفلى للورقة. أما شكل السويق فكان بين الكلوي والدائري في الأنواع المدروسة وعدد الحزم الجانبية في السويق. تم تحليل مصفوفة البيانات عددياً باستخدام البرنامج PAST. التحليل العنقودي للأنواع ونسبة التشابه بينها وضع الأنواع في مجموعتين، الأنواع التي تنتمي إلى جنس *Prunus* شكلت مجموعة واحدة، و *R. damascena* و *P. persica* تنتمي إلى مجموعة واحدة. تشير الدراسة إلى الفائدة التصنيفية للمصدر غير المستغل إلى حد كبير للسمات التشريحية للعائلة الوردية.