COMPARATIVE ANATOMEY OF LEAF AND RACHIS OF ROSA L. (ROSACEAE) IN IRAN AS TAXONOMICAL IMPLICATION

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In this survey, leaf and rachis anatomy of 14 Iranian species of the genus *Rosa* belonging to two subgenera and four sections was studied. The species examined are including: *Rosa persica, R. beggeriana, R. webbiana, R. moschata, R. canina, R. elymaitica, R. orientalis ,R. iberica, R. pulverulenta, R. foetida, R. pimpinellifolia, R. hemisphaerica, R. boissieri* and *R. villosa*. On the basis of morphological features of *R. canina*, eight form-series were distinguished that five of them were studied in this survey. Among 48 anatomical characters examined only 17 characters are valuable at the species level such as presence or absence of fiber in midrib, the number of palisade parenchyma layers, the angle of two parts of blade, presence or absence of spongy parenchyma, shape of auricle in rachis and the distance of auricles.

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تشریح برگچه و محور برگ در گونه های ایرانی جنس گلسرخ به عنوان ابزاری تاکسونومیکی

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در این تحقیق برگچه و محور برگ در١٤ گونه ایرانی جنس گلسرخ متعلق به ٢ زیر جنس و ٤ بخش مورد تشریح مقایسه ای قرار گرفته است. گونه های مورد بررسی عبارتند از:

Rosa elymaitica , R. canina , R. muschata , R. persica, R. webbiana, R. beggeriana, R. pimpinellifolia, R. foetida, R. pulverulenta, R. iberica , R. orientalis, R. villosa , R. boissieri & R. hemisphaeriaca.

بر اساس مطالعات مرفولوژیکی جنس، ٨ گروه در گونه *R. canina* تشخیص داده شد. از بین آنها، ٥ گروه مورد مطالعه تشریحی نیز قرار گرفتند که اختلافاتی را نیز در ساختار تشریحی نشان دادهاند. از میان ٤٨ صفت انتخاب شده ١٧ صفت به عنوان صفات ثابت بین گونه ای در جداسازی گونهها دارای ارزش تاکسونومیکی می باشد مانند: فیبر، تعداد ردیفهای پارانشیم نردبانی، زاویه بین دو نیمه پهنک، پارانشیم اسفنجی، شکل راشی، شکل گوشک ها و فاصله بین دو گوشک.

INTRODUCTION

The genus *Rosa* L. is one of the important genera in the subfamily *Rosoideae*, family *Rosaceae*. The genus *Rosa* comprises four subgenera: *Hulthemia, Platyrhodon, Hesperhodos,* and *Rosa* (Wissemann. & Ritz 2007) and approximately 190 shrubby species distributed widely throughout the temperate and subtropical habitats of the northern hemisphere (Bruneau 2007) From this genus only two subgenera with 13 species and six hybrids (Zielinski 1982) or 14 species and seven hybrids (Khatamsaz, 1992) have been recorded form Iran. Rosa is one of the important

Table 1: Taxonomical status of the genus *Rosa* according to morphological characters in Iran (Zielinski 1982).

Subgenus	Section	Species
Hulthemia	-	*R. persica* Michx. ex Juss.
Rosa	*Pimpinellifoliae* DC.	*R. hemisphaerica* J. Herrmann
		R. foetida J. Herrmann
		R. pimpinellifolia L.
	Cinamomeae DC.	*R. beggeriana* Schrenk
		R. webbiana Wall. ex Royle
	Caninae DC.	*R. boissieri* Crepin
		R. orientalis Dupont ex Ser.
		R. elymaitica Boiss. & Hausskn.
		R. pulverulenta M. B.
		R. iberica Stev.
		R. canina L.
		R. villosa L.
	Synstylae DC.	*R. moschata* J. Herrmann`

elements of Irano-Turanian region. This genus is distributed in most areas of Iran but its main distribution range includes scrublands of southern slopes of Alborz and also Zagros mountains, especially in the north and middle parts of it. Due to wide range of distribution of some species such as R. canina, rather high variation in morphological features is known for this species. Therefore, some authors have recognized several morphological form-series within this species (Nilsson 1972). R. pimpinellifolia another member of the group have a limited distribution which was only known in East Azerbaijan (Zielinski 1982). The genus has been studied from different points including morphology (Zielinski 1982; Wissemann & Ritz 2007), cytology (Akasaka 2003), photochemistry (Mikanagi 2000; Grossi & Raymond 1998) and molecular systematic (Bruneau 2007; Wissemann 2005), but there is only general description of anatomical characters which was mentioned by Metcalfe and Chalk (1957) such as presence of vessels of wood which contain isolated strands of fibers with intervening unlignified parenchyma, vascular bundles separated by narrow primary rays, fibers in secondary phloem, heterogeneous pith, hairs of leaf that are usually unicellular and stalked or sessile capitate glands, and spines that are superficially. Therefore this study aims to 1) present detailed data on the anatomy of the genus and 2) assess this information's value in taxonomy of Rosa.

MATERIALS AND METHODS
In this study, 29 specimens belonging to 14 species and one unknown species (probably *R. hemisphaerica*) are examined. Most specimens were collected directly from the field and some of them were obtained from herbaria, TUH, TARI (acronyms according to Holmgren et al. 1990) and herbarium of Research Center of Agricultural and Natural Resources of Kurdistan. Voucher specimens are listed in the Table 2.

Mature leaflets and rachis were fixed in Ethanol-glycerin (1:1) for 3 months. Cross sections were made at the middle of leaflet and rachis, and stained with methyl green and Bismark brown colors, and then photographed by Leitz light microscope model Wetzlar, Nikon camera model Coolpix S10. For measuring required characters, Measurepro software model HASP 2.17 was used.

RESULTS
Forty six characters have been measured in this study. Of these, only 17 characters including, presence or absence of fiber in midrib, the number of palisade parenchyma layers, the angle between two parts of blade, presence or absence of spongy parenchyma, presence or absence of auricle in rachis and the distance between two auricles of rachis are the most important ones for separating of the species examined.

General characteristics
Blade. Blade includes two distinct forms with angle variability from $70°$ (Fig. 4b) to $180°$ (Fig. 22a). Upper epidermis consists of thin or thick cuticle (*R. elymaitica*, Fig. 12b). Regarding mesophyll, most of species have palisade parenchyma layer in upper surface with two or rarely three (Fig. 11b) layers and spongy parenchyma layer in lower surface (dorsiventral mesophyll) while a few species i. e. *R. persica* (Fig. 22b), *R. hemisphaerica* (Fig. 3c) and *R. pimpinellifolia* (Fig. 5c) have three (Fig. 11b) to six (Fig. 22b) layers of palisade parenchyma and lack of spongy layer (isobilateral mesophyll). Considering presence of hair on midrib, all studied species have hairs except in a few species such as *R. webbiana* (Figs. 8a, 8b)*, R. elymaitica* (Figs.12a, 12b) and *R. pimpinellifolia* (Figs. 4a, b; 5a, b). According to presence of fiber around the phloem of midrib, the species of *Rosa* are divided into two groups: a) with fiber: *R. elymaitica* (Fig. 12a), *R*

Table 2. The most important anatomical characters measured for the examined *Rosa* species.

Midrib							Blade			Rachis								
Species	H /g	L.E.C.W/ U.E.C.W (µm)	L/W of ph (µm)	L/W of x (µm)	P.T.Ph(µm)	A.B	UP. LTh (µm)	LP. LTh (µm)	S.C.Th (µm)	L/W of R (µm)	Au. Co. Th (µm)	Dis. Au (µm)	L/W of Au (µm)	L/W of Ph (µm)	L/W of X (µm)	H	G	Co. Th (µm)
R. moschata	s/g	30/35	160/30	120/80	Fiber 16	95°	2/60	–	50	670/710	34 +	400	97/80	250/47	220/150	S+/	–	30
R. foetida	s	50-30/20-30	120-220/24-40	120-220/40-90	–	130-160	2/3 60-80	–	26-70	650-750/700-1000	+/ 130-200	140-540	30-100/90	260-530/70	200-500/140-230	S/+	+	50-70
R. hemisphaerica	s	40/30	210/70	180/130	–	130	5-6 120	100	-	700/700	+ 130	400	100-700/140	300/50	240/180	S+/	–	40
R. pimpinellifolia	–	28-68/35-55	13-42/18-46	10-32/50-60		70-150	5-6 100	60	-	600-650/600-650	+ 36-42	340-400	100-120/100-120	240-250/27-40	210-220/130-170	–	–	30-40
R. webbiana	-	72/27	230/35	180/99		125	2-50	–	48	1. 3/970	71 +	310	102/110-	500/50	450/200	-	-	90
R. beggeriana	s/g	-	160-230/40-60	100-160/70-100	–	110-140	2-3 50-100	–	20-40	930-950/850-870	–	–	0	230-430/31-40	140-350/200-130	S+/	+	30-90
R. boissieri	+ s	70-90/40-50	210-260/30-50	180-220/110-160	fiber 16	95	2-3 60-80-100	–	70	850-900/900-100	+ 100-160	110-400	70-120/120	280-470/40-60	240-350/210	S+/-	-	40-50
R. pulverulenta	+ - s/g	20-45-/30	150-230/25-60	170-120/70.	–	98-95°	68-80 2-3	–	48-58	600-650/650	+ 68-70	260-360	60--70/70-100	310-530/50-90	200-400/160-250	S-/+	+	40-50
R . iberica	+ g/s	20-30/30-45	190-230/70	190-230/20-50	–	95-100	2 60-67	–	40-50	850-1100 750-1050	44-59 +	350-400	130-160	450-550/50-100	400-500 170-200	+ S	+	30-40
R. elymaitica	-	30/20	100/20	100/70	10 fiber	150°	2 187	–	138	530// 570	+ 100	310	43/120	220/50	210/170	-	-	25
R. orientalis	s +	10--29/10-29	53-200/10-60	43-140/30-70	14 fiber	110	2-3 80-100	–	58-68	600-700	-/+	300-500	50-100/120-	240--470/47-53	210/410	S+	+	20-40
R. villosa	+ s	45/66	230/40	170/110	fiber 20	160-170°	2 60-70	–	73	750/700	+ 29	260	37/72	360/42	320/130	S+	+	59
R. canina-A 7395	-	36-46	190/40	150/100	–	140-150	2 100	–	70	750/900	81	500-600	80/103	400/71	300/190	-	-	32
R. canina-C 37211	+ s	40-60/45	200/44	170/110	–	100	2 66	–	45	700/650	41	69-100	69/100	270/50	210/160	S+		50
R. canina-D 37303	+ s	40/-45	270/39	190/120	–	80	2 80	–	50	870/920	+ 50	430	110/98	340/57	350/210	+ S	–	67
R. canina-E 37407	+ s	34/45	280/390	240/150	–	110	2 45	–	48	670/730	+ 82	330	570/71	320/50	280/130	S+	–	38
R. canina-F 37401	–	34/43	180/35	120/75	Fiber 16	80-85	2-3 69	–	90	650/650	+ 55	300	130/120	220/54	190/180	–	–	50
R. SP. /	-	57/20	180/40	128/80	Fiber 10	120-125°	2-3 56	–	30	340/460	+ 64	260		250/30	210/110	/+ S	–	20
R. persica	+ s	42/53	150/37	120/76	–	180	6/300	–	–	–	–	–	–	–	–	–	––	-—

Abbreviations: H/g: Hair/gland; L.E.C.W/U.E.C.W: Lower epidermis collenchyma width/ upper epidermis collenchyma width; L/W of ph :Length/width of phloem; L/W of X: Length/width of xlem; P.T.Ph: Protective tissue of phloem; A.B:Angle of Blade; UP.LTh: Upper palisade thickness; LP.LTh: Lower palisade thickness; S.C.Th; Spongy thickness; L/W of R:Length/ width of rachis; Au.Co.Th: Auricle collenchyma thickness; Dis.Au: Distance of auricle; L/W of Au: Length/ width of auricle; Ph: Phloem; X: Xylem; Co.Th: Collenchyma thickness; s: simple; g: gland; +: Presence; -: Absence.

moschata (Fig. 20b), *R. villosa* (Fig. 13b), *R. orientalis* (Fig. 15a), *R. canina*-F (Fig. 21a) and b) without fiber: *R. boissieri* (Fig. 11a), *R. pulverulenta* (Figs. 10a, b), *R. beggeriana* (Figs. 6a, 6b-7a), *R. foetida* (Figs. 1b, 2b), *R. hemisphaerica* (Fig. 3b), *R. iberica* (Fig. 11a*), R. pimpinellifolia* (Figs. 4b, 5b), *R. webbiana* (Fig. 8b)*, R. persica* (Fig. 22a), *R. canina*-A (Fig. 14b), *R. canina*-C (Fig. 17b), *R. canina*-D (Fig. 18d) and *R. canina*-E (Fig. 19b). Two other important separating characters in this study belong to presence of internal phloem and hypodermis that except in *R. pimpinellifolia* (Fig. 5b) and *R. persica* (Fig. 22b) are absent in the genus, respectively. In all studied species, we observed crystal of calcium oxalate in midrib and blade but the number of them is different among them.

Rachis. Shape of epidermal cells ranges from orbicular (Fig. 6d), oblong (Fig. 11c) to square (Fig. 1d), collenchyma layer thickness ranges from 20 µm (unknown specimen, see Table 2) to 90 µm (in *R. webbiana*). With regard to vascular bundle, there is a main vascular bundle. However, two (Figs. 1d, 2d, 3d, 4d) or rarely four (Fig.11c) subordinate bundles are recognized. In the case of auricle, most specimens have two remarkable auricles except in a few specimens such as *R. beggeriana* (Figs. 6d) and a population of *R. orientalis* (Fig. 16c), angle between auricles and horizontal axis is variable (see figures of rachis), distance between two auricles ranges from 69 µm (Fig. 17d) to 600 µm (Fig. 14d). On the basis of presence of hairs on rachis, the species of *Rosa* are divided into two groups: a) rachis glabrous: *R. webbiana* (Fig. 8d), *R. elymaitica* (Fig. 12c), *R. pimpinellifolia* (Figs. 4d, 5d), *R. canina*-F (Fig. 21c) and *R. canina*-A (Fig. 14d) and b) rachis hairy with glandular hairs that includes all other studied species. One of the most important characters is the ratio of length to width of rachis (L/W ratio), the smallest ratio (0.92µm) is observed in *R. elymaitica* (Fig. 12c) and the largest one (1.3 µm) belongs to *R. webbiana* (Fig. 8d). Regarding to collenchyma thickness of auricle, the smallest thickness (20 µm) is observed in *R. orientalis* (Fig.15c) and the largest is in *R. webbiana* (Fig. 8d).

DISCUSSION

Morphologically the genus, Rosa has been divided into four subgenera of which only two subgenera, Hulthemia and Rosa are distributed in Iran.

Subgenus *Hulthemia* Dum.

This subgenus consists of only one species, *R. persica*. Regarding to morphological characters, this species is completely different from all other species of *Rosa* by having bushy habit, simple and estipulate leaf and yellow flower with purple blotch at the base of petal (Zielinski 1982). Interestingly, anatomical characrers also completely separate this species from the other species by the following characters: angle 180° between two halves of the blade (Fig. 22a), presence of hypodermis in the blade (Fig. 22b), lacking of spongy parenchyma layer (isobilateral mesophyll) (Fig. 22b) and having the highest thickness (300 µm) and number (six layers) of palisade parenchyma layers (Figs. 22a, b). Boissier (1872), distinguished this subgenus as a separate genus, *Hulthemia* Dum. Presence of above mentioned diagnostic characters support this classification to some extent.

Subgenus *Rosa*
Section *Pimpinellifoliae* DC.
According to morphological traits, this section has flowers without bracts, high number of small, round leaflets, and intensive colored, often black hips (Wissemann. and Ritz. 2005).This section includes, *R. foetida, R. hemisphaerica* and *R. pimpinellifolia* in Iran. According to anatomical characters, these species have some common features as: absence of fiber around phloem of midrib (Figs. 1b, 2b, 3b, 4b, 5b), angle between two halves of the blade that ranges from 80°-160°(Figs.1a, 2a, 3a, 4a, 5a). On the other hand, these species have some features that are different among them such as lacking of hair on midrib and rachis of *R. pimpinellifolia* (Figs .4a,d; 5a,b), presence of internal phloem in midrib and the smallest L/W ratio (see column L/W of Au in Table 2) of auricle in *R. foetida* (Figs. 1d and 2d) and absence of spongy parenchyma layer in *R. hemisphaerica* (Fig. 3c) and *R. pimpinellifolia* (Figs. 4c and 5c) (isobilateral mesophyll) in comparison *R. fotida* (dorsiventral mesophyll). Interestingly, presence of the last mentioned character (isobilateral mesophyll) is observed only in two mentioned species and also *R. persica* that makes these species close to *R. persica*. Moreover according to anatomical studies, these species are separated to some extent.

Section *Cinnamomeae* DC.
Section *Cinamomeae* is the largest section of subgenus *Rosa* in the world that only includes *R. beggeriana* and *R. webbiana* in Iran. Regarding morphology, this section has narrow and thin stipule with straight auricle, flowers with bracts and simple sepals (Zielinski 1982). With respect to anatomical traits, these species have some separating characters that the main ones are as follows: presence of simple and glandular hairs on rachis and midrib of *R. beggeriana* (Figs. 6b, 6d, 6c, 7a, 7b and 7c) and absence of auricle on its rachis (Fig. 6d) in comparison *R. webbiana* (Figs. 8b, 8c, 8d).

Therefore, these species are well separated by anatomical evidences.

Section *Synstylae* DC.
This section has a unique diagnostic morphological feature, the agglutinated (united in length) style (Zielinski 1982). It consists of only one species, *Rosa moschata* (cultivated in some places) in Iran. On the basis of presence of fiber around phloem of midrib, this section is separated from sections, *Cinnamomeae* and *Pimpinellifolia* ; also, on the basis of this character a close relationship between this section and section *Caninae* can be inferred.

Section *Caninae* DC.
This section is a heterogeneous group and can not be characterized by specific morphological traits, however presence of branched (specifically in *R. canina*) or simple and also deciduous (specifically in *R. canina* and *R. iberica*) or persistent sepals are important characters of the section (Zielinski 1982). In cytological view, this section is recognized by a unique meiotic system that in which because of inheritance of 4/5 of the genome by the egg cell in pentaploid individuals leads to a pronounced matroclinal inheritance that is so-called canina meiosis (Bruneau 2007). This section is the largest one in Iran and includes *R. elymaitica, R. canina, R. villosa, R. iberica, R. pulverulenta, R. boissieri* and *R. orientalis*. Some of the important characters of this section are as follows; ʻpresence of dorsiventral mesophyll in all species, presence of hairs on midrib and rachis (except *R. elymaitica* (Figs. 12a-12d) and *R. canina-A* (Figs. 14a-14d) and *R. canina-F* (Figs. 21a-21c) and also presence of fiber around phloem of midrib (except *R. elymaitica*) (Fig. 12a), *R. villosa* (Fig. 13b) and *R. orientalis* (Fig. 15a). Regarding the thickness of upper cuticle, the thickest one is observed in *R. elymaitica* (Fig. 12b). Related to *R. canina* because of high variation in morphological characters, Nilsson (1972) recognized four form-series within the species. Based on the variation of indumentum of leaflets and pedicel in several individuals of the species in Iran, eight form-series were recognized that five of which were included in this work. In view of anatomy, except of presence of hairs on midrib and rachis (*R. canina-* C, D and E) and fiber around phloem of midrib (*R. canina-* F) in some form-series, most characters are more or less similar in these form-series. As is concluded from anatomical evidence, this section is more heterogeneous in comparison than the other sections. At the end, it should be noted that anatomical characters are useful for separating species while is less useful for isolating sections.

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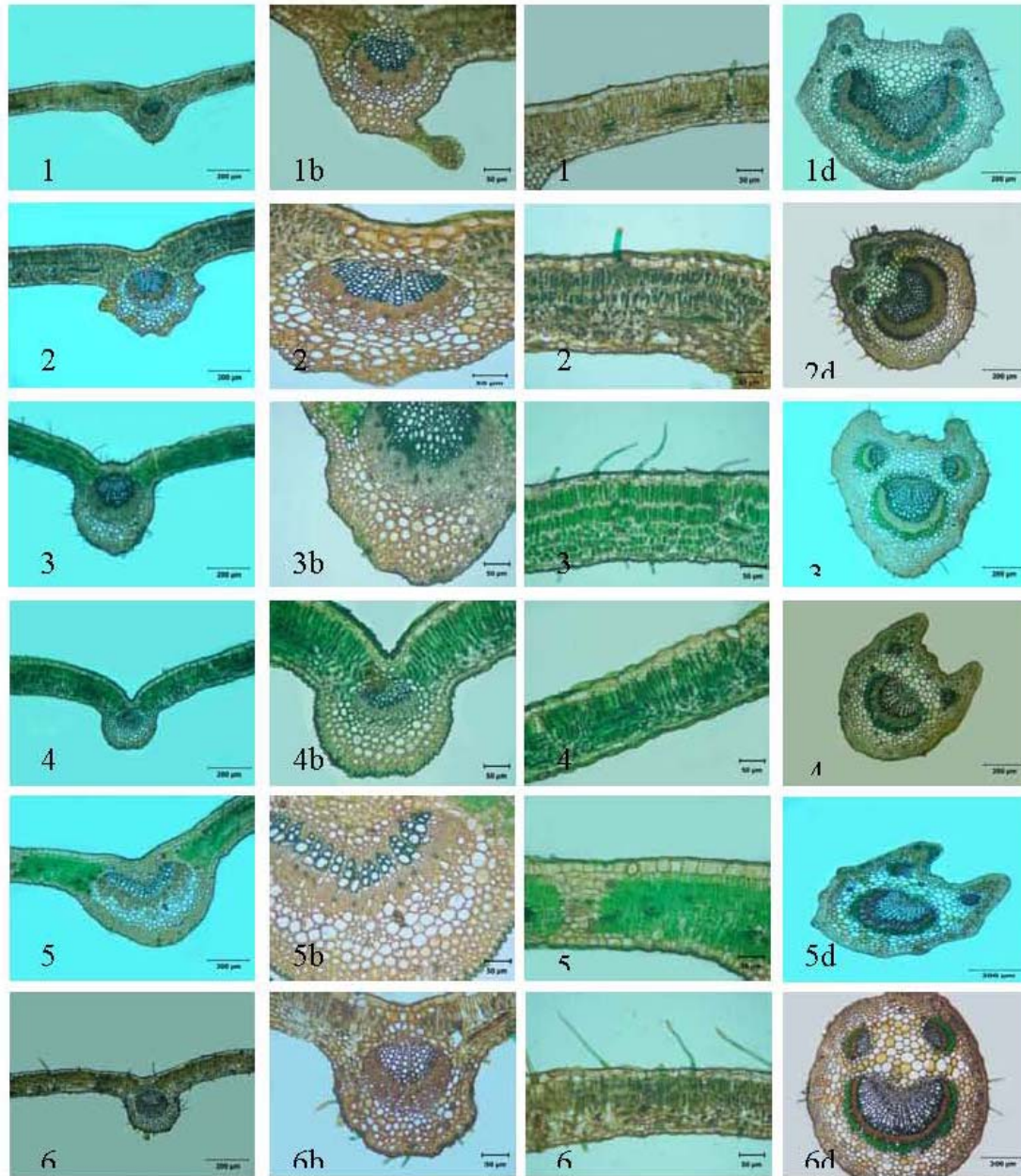
Figs. 1-6: Blade (a, b, and c) and midrib (d) in *Rosa* species. 1a, b, c and d, *R. foetida* (37165); 2 a, b, c and d, *R. foetida* (37157); 3 a, b, c and d, *R. hemisphaerica*; 4 a, b, c and d, *R. pimpinellifolia* (37143); 5a, b, c and d, *R. Pimpinellifolia* (37681); 6 a, b, c and d*, R. beggeriana* (37306).

Figs. 7-12: Blade (7a-b, 8a-c, 9a-c, 10a-c, 11a-b and 12a-b) and midrib (7c, 8d, 9d, 10d, 11d and 12d) of *Rosa* species. 7a, b, c and d, *R. beggeriana* (37396); 8a, b, c & d, *R. webbiana* (50764); 9a, b, c and d, *R. iberica* (37313); 10a, b, c and d, *R. pulverulenta* (37155); 11a, b and c, *R. boissieri* (1990); 12a, b and c, *R. elymaitica* (20063).
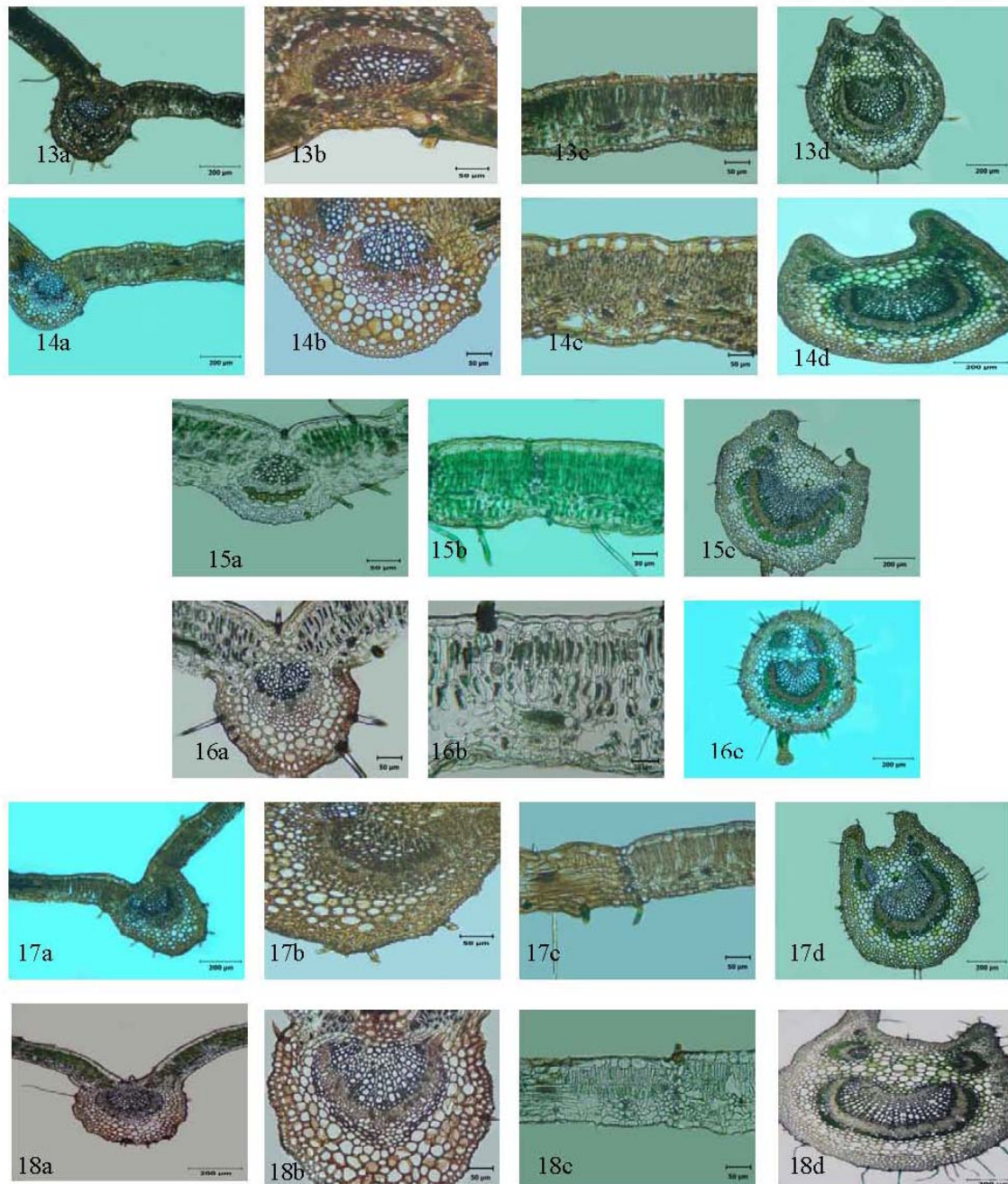
Figs. 13-18: Blade (13a-c, 14a-c, 15a-b, 16a-b, 17a-c and 18a-c) and midrib (13d, 14d, 15c, 16c, 17d and 18d) in *Rosa* species. 13a, b, c and d, *R villosa* (24175); 14a, b, c and d, *R. canina* A(37395); 15 a, b and c, *R. orientalis* (5792); 16a, b and c, *R. orientalis* (25850); 17 a, b, c and d, *R. canina* C (37211); 18a, b, c and d, *R. canina* D (37303).

Figs. 19-23: Blade (19a-c, 20a-c, 21a-b, 22a-b and 23a-b) and midrib (19d, 20 d, 21c and 23c) 19a, b, c and d, *R. Canina* E (37407); 20a, b, c and d, *R. moschata* ; 21a, b and c, *R. Canina* F (37401); 22° and b, *R. persica*; 23a, b and c, *R.* sp.