FOLIAR ANATOMY OF SOME SALIX SPECIES (SALICACEAE) IN IRAN

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Received 13 06 2010. Accepted for publication 31 10 2010.

Khalili, Z., Maassoumi, A. A., Ghahremaninejad, F. & Mirzaie-Nodoushan, H. 2010 12 31: Foliar anatomy of some *Salix* species (*Salicaceae*) IQ, UDQ ± *Iran. J. Bot. 16 (2): 293-302.* Tehran.

Foliar anatomy of nine species of genus *Salix* belonging to different subgenera including *Salix aegyptiaca, S. caprea, S. cinerea, S. elbursensis, S. euxina, S. issatissensis, S. triandra, S. wilhelmsiana,* and *S. zygostemon* (*S. cinerea* × *S. elbursensis*) were studied using light microscope. Several features of leaf anatomy, i. e. transverse section outline, lamina thickness, epidermis characteristics, hypodermis existence, mesophyll features, crystal types, vascular bundles characteristics, etc. are discussed here. Several anatomical characters in this study confirm the infrageneric classification of *Salix*. On the base of presence or absence of hypodermis layer in lamina, the genus *Salix* can be divided into two types. The outline of the transverse sections and stomata type differ among sect. *Cinerella* and the other sections. The delimitation of the closely related species of *S. aegyptiaca, S. caprea and S. cinerea* belong to sect. *Cinerella* is difficult, but they can be distinguished based on some anatomical characters. This study has provided interesting results about the studied hybrid *S. zygostemon*.

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Key words. Anatomy, *Salix*, infrageneric taxa, Iran.

آناتومی برگ برخی از گونه‌های بید (تیره بید) در ایران

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در این پژوهش، مطالعه آناتومی برگ نه گونه از جنس بید متعلق به دو زیر جنس مختلف با استفاده از میکروسکوپ نوری انجام گرفت که عبارتند از:

Salix aegyptiaca L., *S. caprea* L., *S. cinerea* L., *S. elbursensis* Boiss., *S. euxina* I. V. Belyaeva, *S. issatissensis* Maassoumi, Moeeni & Rahiminejad, *S. triandra* L., *S. wilhelmsiana* M. Beib., *S. zygostemon* Boiss. (*S. cinerea* × *S. elbursensis*)

صفات تشریحی مختلفی مورد بررسی قرار گرفت که شامل موارد زیر است: شکل کلی برش عرضی برگ، ضخامت پهنک، ویژگی‌های اپیدرم، وجود هیپودرم، صفات مزوفیل، انواع کریستال، ویژگی‌های دستجات آوندی و غیره. صفات تشریحی برگ در این گونه‌ها، تقسیم‌بندی گونه‌های بید در زیرجنس و بخش‌ها را تائید می‌کند. جنس بید بر اساس وجود یا عدم وجود لایه هیپودرم در پهنک برگ به دو تیپ تقسیم می‌شود. در گونه‌های متعلق به بخش *Cinerella*، شکل کلی برش عرضی برگ و نوع روزنه با گونه‌های متعلق به سایر بخش‌ها متفاوت است. شناسایی و تشخیص گونه‌های *S. aegyptiaca* , *S. caprea* و *S. cinerea* متعلق به بخش *Cinerella* مشکل بوده ولی مطالعات تشریحی برگ تشخیص این گونه‌ها را آسان می‌کند. مطالعات آناتومی برگ، در این پژوهش نتایج قابل توجهی در رابطه با گونه دورگه *S. zygostemon* آشکار می‌سازد.

INTRODUCTION

The role of anatomical data in traditional taxonomy has been recognized since the variation within a species, genus or family is usually reflected in anatomical features as well. The comparative anatomy of leaves has also shown to be of considerable significance in taxonomy by several workers such as Hagerup (1953), Metcalfe & Chalk (1957), Hickey (1973), Cutler (1984), Stebbins & Khuscht (1961), and Afolayan & Meyer (1995). Little microscopic details have been published on the anatomy of *Salix* L. genus apart from the work of Metcalfe & Chalk (1957) on the family *Salicaceae*. This family was divided into *Salix* and *Populus* when it was originally described by Linnaeus (1753). *Salix* is by far the larger of the two genera of the family (Azuma *et al.*, 2000). The species of the genus *Salix* are deciduous trees and shrubs with simple, stipulate leaves alternately arranged on woody stems. Based on several publications (Fang-Zhen 1987; Skvortsov 1999; Argus 1997, 2007; Ohashi, 2000) about 526 distinct species are recognized for the genus worldwide. Former Soviet Union includes 120 species, the New World 103, China 275, Europe 65, Pakistan 26 and Iran 31 species and 6 hybrids (Maassoumi 2009). According to the latter work, the *Salix* species are subdivided into three subgenera: Subgen. *Protitea*, *Salix*, and *Vetrix*.

MATERIALS AND METHODS

Based on a project for the establishment of living collections of the Iranian species of the genus *Salix* in Karaj and also several other cities of the country, the collection of living materials was found by Maassoumi and colleagues in the Research Institute of Forests and Rangelands from 1996. Fresh leaf materials including the largest one in branchlets were collected from collection and fixed in FAA and stored in 70% alcohol. Transverse sections of leaf were prepared by hand cutting. Sections were cleared with Sodium hypochlorite, dehydrated and stained by Methyl Blue and Carmino-Vest and mounted in Glycerin. After preparing the slides, they were photographed by different magnifications of ZEISS Standard 20 light microscope and Canon camera model G10. Voucher specimens are preserved in Tarbiat Moallem University herbarium (FAR). List of the studied species is presented in Table 1. The measurements carried out on average 100 transverse sections.

RESULTS

Results revealed a number of interesting features, which are given in Tables 2, and 3. It is found that anatomical study may provide useful characters for classification and distinguishing species in the genus.

Leaf lamina TS

The outline of the transverse sections of lamina exhibited flat shape, less height in midrib, except in *S. aegyptiaca*, *S. caprea*, and *S. cinerea* that were round U- shape (Figs. 1b, 2b & 3b).

Lamina

Lamina mostly had a regular shape and arrangement. The following layers could be seen from up to down respectively: trichomes, cuticle layer, upper epidermis, palisade parenchyma, spongy parenchyma, lower epidermis, cuticle layer, and trichomes. There are some exceptions such as the existence of lower palisade parenchyma, and a single lower hypodermis layer.

Lamina thickness. The lamina thickness was measured by an average thickness of between 0.13 to 0.22 mm. *S. caprea* had the thinnest leaf, and *S. issatissensis* and *S. zygostemon* had the thickest one.

Epidermis. The epidermis, which normally consists of a single layer of the cells forms outermost part of the leaf.

In the studied species, epidermis is one layered. The cells are variable in size and shape, which may be rounded or rarely rectangular. The cells of upper surface are usually larger than those in the lower surface.

S. triandra has the largest upper epidermis cells. The average height of the upper epidermal layer was 0.021 mm, and the thinnest ones in upper epidermis layer was *S. euxina* with 0.011 mm. *S. caprea* with 0.001 mm height in the lower epidermis layer was the thinnest and *S. elbursensis* with 0.01 mm is the thickest one. The cells shape was mostly rectangular. Cuticle thickness and trichomes density in upper epidermis were more than that of lower epidermis in all of the studied species. Trichomes were numerous on the midrib.

Hypodermis. It is suggested that there is sometimes a distinct layer immediately adjacent to the epidermis, the hypodermis, consisting either of transparent parenchymatous cells or, more rarely, of fibrous cells (Metcalfe & Chalk 1957). It is found hypodermis within the lower epidermis in *S. euxina*, *S. issatissensis*, and *S. triandra* (Figs. 5a, 6a &7a).

Stomata. Transverse sections of stomata revealed that the stomata in *Salix* species are superficial, present on both surfaces, mostly hypoamphistomatic but hypostomatic in *S. aegyptiaca*, *S. caprea* and *S. cinerea*.

Table 1: List of *Salix* species used in this study.

Subgenus	Section	Species	Origin	Voucher specimens [FAR]
Salix	*Salix*	*S. euxina* I. V. Belyaeva	Ardebil	Kh. 8748
Salix	*Salix*	*S. issatissensis* Maassoumi, Moeeni & Rahiminejad	Khorasan	Kh. 8742
Salix	*Triandrae* Dumort.	*S. triandra* L.	Markazi, E. Azarbaijan	Kh. 8720, Kh. 8752
Vetrix	*Cinerella* Duby in DC.	*S. aegyptiaca* L.	Tehran, Kerman	Kh. 8715, Kh. 8731
Vetrix	*Cinerella* Duby in DC.	*S. caprea* L.	W. Azerbaijan, E. Azerbaijan	Kh. 8736, Kh. 8704
Vetrix	*Cinerella* Duby in DC.	*S. cinerea* L.	Chaharmahal & Bakhtiari, Markazi	Kh. 8702, Kh. 8729
Vetrix	*Helix* Dumort.	*S. elbursensis* Boiss.	Tehran	Kh. 8721, Kh. 8707
Vetrix	*Cheilophilae* Hao**.**	*S. wilhemsiana* M. Bieb.	Chaharmahal & Bakhtiari	Kh. 8725, Kh. 8747
		S. zygostemon Boiss.	Chaharmahal & Bakhtiari, W. Azerbaijan	Kh. 8727, Kh. 8728

Table 2: Lamina characteristics of *Salix* species.

La.: Leaf anatomy(B: Bisacial, I2: Isobilateral 2-layered), **St.:** Stomata type (1: Hypoamphistomatic, 2: Hypostomatic) **Lth.:** Lamina thickness**, Cth.:** Cuticle thickness**, Hyth.:** Hypoderm thickness**, Ppln.:** Palisade parenchyma layer number**, Spln.:** Spongy parenchyma layer number, **Ppcwsh**.: Palisade parenchyma cell wall shape (1= Straight, 2= Substraight, 3=Crinkle) **Spcsh**.: Spongy parenchyma cell shape(1=Short & undulate, 2=irregular), **Pprat**.: Palisade parenchyma ratio, **Spcsh**.: Spongy parenchyma cell shape, **Pprat**.: Palisade parenchyma ratio, **Ueth**.: Upper epidermis thickness, **Leth**.: Lower epidermis thickness, **Ct**.: Crystal type (1=Clustred, 2= Prismatic crystals, 3= Crystal sands).

	Lamina (T. S.)												
Taxon	La.	St.	Lth. (mm)	Cth.	Hyth. (mm)	Ppln.	Spln.	Ppcwsh.	Spcsh.	Pprat.	Ueth. (mm)	Leth. (mm)	Ct.
S. aegyptiaca	B	2	0.1(0.14) 0.15	Ad>Ab	-	2	2-3	1	2	2(2.7)3.3	0.011(0.014) 0.02	0.008(0.009) 0.012	1,2,3
S. caprea	B	2	0.12(0.13) 0.15	Ad>Ab	-	2	2-3	3	2	2(2.6)3.3	0.012(0.017) 0.022	0.001(0.007) 0.017	1,2,3
S. cinerea	B	2	0.14(0.15) 0.17	Ad>Ab	-	2	2-3	1	2	1.1(2.1)2 .7	0.013(0.016) 0.022	0.009(0.012) 0.016	1,2,3
S. elbursensis	B	1	0.14(0.18) 0.22	Ad>Ab	-	2	3	3	1	1.1(1.6)1 .9	0.012(0.014) 0.017	0.007(0.01) 0.012	1,2,3
S. euxina	I2	1	0.11(0.18) 0.2	Ad≈Ab	0.01(0.012) 0.015	2=2	-	2	-	1.4(1.8)2 .3	0.01(0.011) 0.015	0.01(0.011) 0.012	1,2,3
S. issatissensis	I2	1	0.21(0.22) 0.24	Ab>Ad	0.012(0.015) 0.017	2=2	-	1	-	1.2(1.4)1 .5	0.015(0.019) 0.022	0.012(0.015) 0.017	1,2,3
S. triandra	I2	1	0.17(0.21) 0.24	Ad>Ab	0.012(0.015) 0.017	2=2	-	2	-	1(1.2)1.1	0.017(0.021) 0.027	0.01(0.013) 0.017	1,2,3
S. wilhelmsiana	I2	1	0.16(0.20) 0.23	Ad>>> Ab	-	2=2	-	1	-	1(1.1)1.4	0.015(0.021) 0.025	0.017(0.019) 0.022	1,2,3
S. zygostemon	B	1	0.2(0.22) 0.25	Ad>Ab	-	2	2	1	1	1.6(2.1)2 .8	0.015(0.019) 0.025	0.01(0.012) 0.015	1,2,3

Table 3: Midrib characteristics of *Salix* species.
Mth.: Midrib thickness, **Ucth.:** Upper cortex thickness, **Lcth**.: Lower cortex thickness, **Ucc.:** Upper collenchymas cells (A: Annular), **Lcc**.: Lower collenchymas cells (A: Annular). **Uccn**.: Upper collenchymas cells number, **Lccn**.: Lower collenchymas cells number, **Upsh**.: Upper parenchyma cells shape (1=Orbicular, 2=Suborbicular, 3= hexagonal 4=irregular) **Lpsh**.: Lower parenchyma cells shape(1=Orbicular, 2=Suborbicular, 3= hexagonal 4=irregular), **Upn**.: Upper parenchyma cells number, **Lpn**.: Lower parenchyma cells number, **Uscth**.: Upper schlerenchyma thickness, **Lscth**.: Lower schlerenchyms thickness, **Vbn**.: Vascular bundle number, **Ct**.: Crystal type(1=Clustered, 2= Semi-clustered 3=Prismatic crystals, 4=Crystal sands).

	Midrib														
Taxon	Mth. (mm)	Ucth. (mm)	Lcth. (mm)	Ucc.	Lcc.	Uccn.	Lccn.	Upsh.	Lpsh.	Upn.	Lpn.	Uscth. (mm)	Lscth. (mm)	Vbn.	Ct.
S. aegyptiaca	0.7(0.93)1.1	0.19(0.24) 0.25	0.20(0.24) 0.27	A	A	3-5	3	1,2,3,4	1,2,3,4	5-6	5-6	0.03(0.04) 0.05	0.04(0.05) 0.07	1(2)	1,2,3.4
S. caprea	0.53(0.73)1	0.07(0.15) 0.16	0.1(0.18) 0.25	A	A	2-3	2-3	1,2,3,4	1,2,3,4	2-3	3-4	0.02(0.04) 0.05	0.04(0.06) 0.07	1(2)	1, 3, 4
S. cinerea	0.84(1)1.1	0.17(0.18) 0.23	0.2(0.23) 0.25	A	A	3-4	3-4	1,2,3,4	1,2,3,4	5-6	6-8	0.02(0.03) 0.04	0.04(0.05) 0.06	1	1, 3, 4
S. elbursensis	0.35(0.39) 0.47	0.05(0.06) 0.07	0.08(0.1)0.11	A	A	1	1-2	1,2	1,2,4	3	4-5	0.01(0.01) 0.025	0.017(0.02) 0.025	1-2	1, 3, 4
S. euxina	0.37(0.48) 0.55	0.06(0.09) 0.11	0.07(0.1) 0.11	A	A	2-3	2-3	1,2	1 2	3	3-4	0.02(0.02) 0.025	0.01(0.03) 0.04	1(2)	1, 3, 4
S. issatissensis	0.83(0.85) 0.90	0.13(0.14) 0.16	0.22(0.23) 0.23	A	A	4-5	3	1,2,4	1,2,4	3	4-5	0.06(0.06) 0.07	0.07(0.07) 0.08	1	1, 3, 4
S. triandra	0.45(0.66) 0.82	0.09(0.1) 0.12	0.12(0.14) 0.16	A	A	3-4	(2)3	1,2,4	1,2,4	3-4	4-5	0.02(0.025) 0.027	0.03(0.04) 0.06	1	1, 3, 4
S. wilhelmsiana	0.29(0.36) 0.42	0.05(0.06) 0.09	0.09(0.11) 0.13	A	A	1	1-2	1,2	1,2,4	2-3	3-4	0.025(0.03) 0.035	0.025(0.03) 0.035	1	1, 3, 4
S. zygostemon	0.73(0.76) 0.95	0.13(0.15) 0.17	0.17(0.19) 0.22	A	A	3(4)	3(4)	1,2,3,4	1,2,3,4	4	5	0.03(0.04) 0.05	0.05(0.06) 0.07	1(2)	1, 3, 4

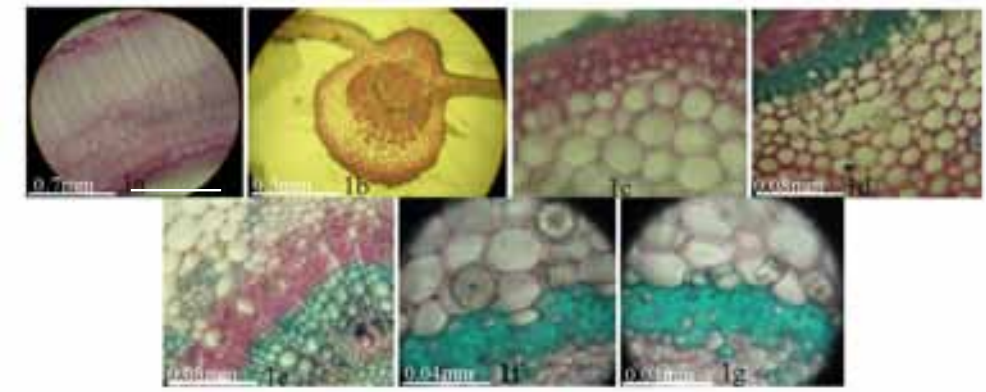

Fig. 1. Leaf transversal sections of *salix aegyptiaca*: (a) the lamina with palisade parenchyma in upper surface and spongy parenchyma in lower surface; (b-e) the midrib includes epidermis, collenchymas, parenchyma, and vascular tissues; (f-g) the crystals.

Fig. 2. *Salix caprea*: (a) the lamina includes palisade parenchyma with crinkle cell walls in upper surface and spongy parenchyma in lower surface; (b-d) the midrib; (e) the crystals.

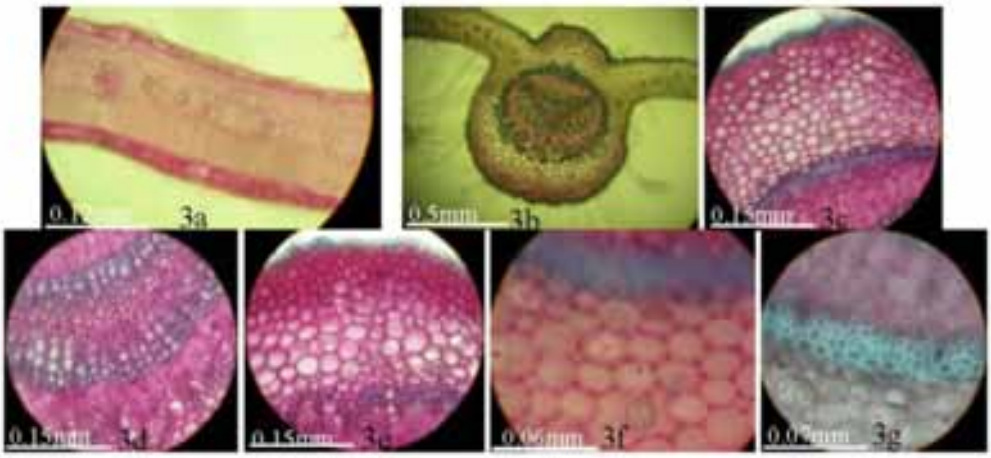

Fig. 3. *Salix cinerea*: (a) the lamina with palisade parenchyma in upper surface and spongy parenchyma in lower surface; (b-e) the midrib; (f-g) the crystals.

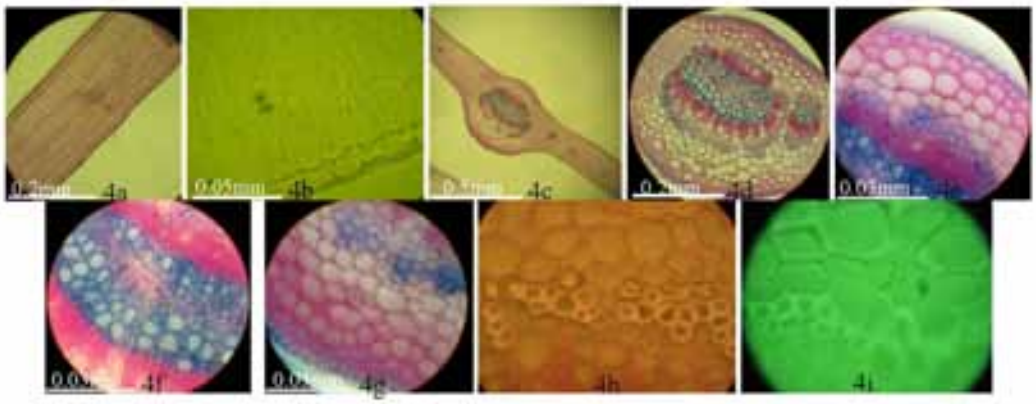

Fig. 4. *Salix elbursensis*: (a-b) the lamina includes palisade parenchyma with crinkle cell walls in upper surface and spongy parenchyma in lower surface; (c-g) the midrib; (h-i) the crystals.

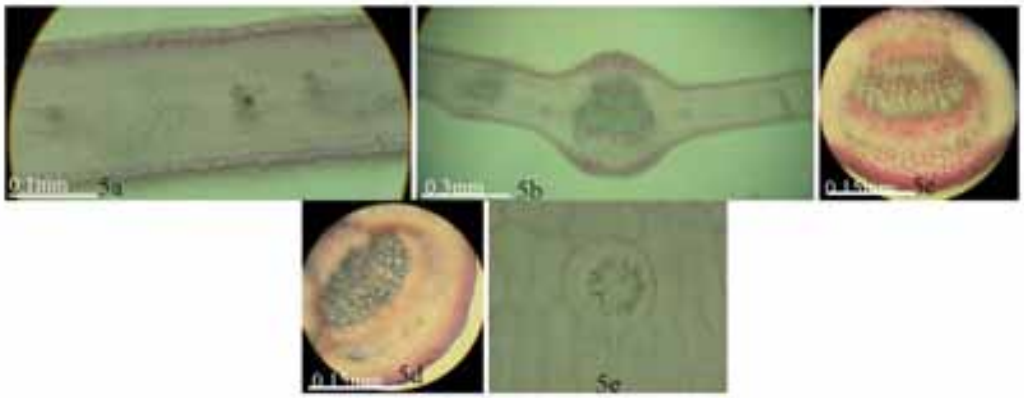

Fig. 5. *Salix euxina*: (a) the lamina includes hypodermis in lower surface and palisade parenchyma in both surfaces; (b-d) the midrib; (e) the crystals.

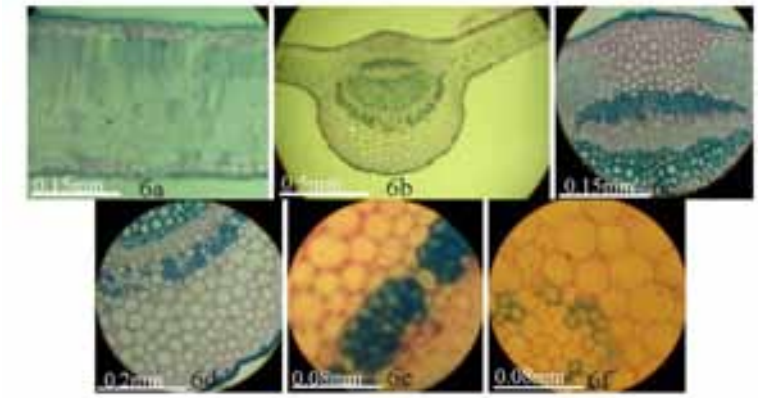

Fig. 6. *Salix issatissensis*: (a) the lamina includes hypodermis in lower surface and palisade parenchyma in both surfaces; (b-d) the midrib; (e-f) the crystals.

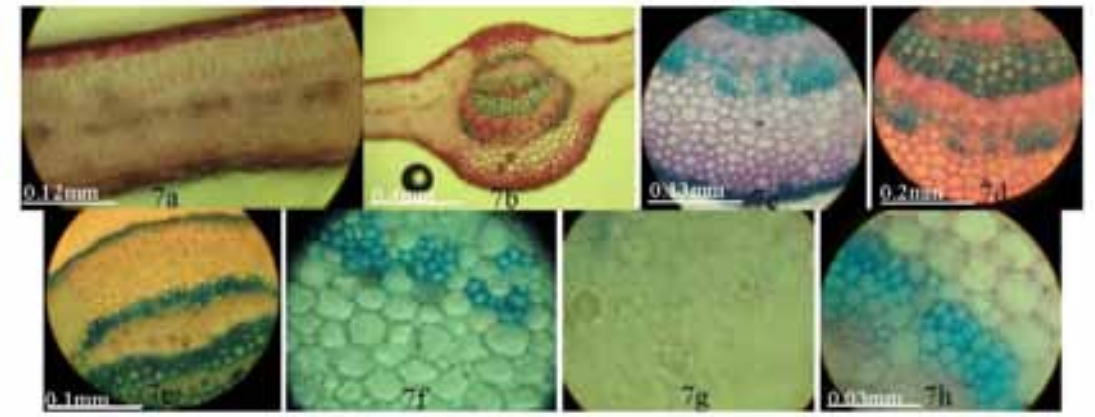

Fig. 7. *Salix triandra*: (a) the lamina includes hypodermis in lower surface and palisade parenchyma in both surfaces; (b-e) the midrib; (f-h) the crystals.

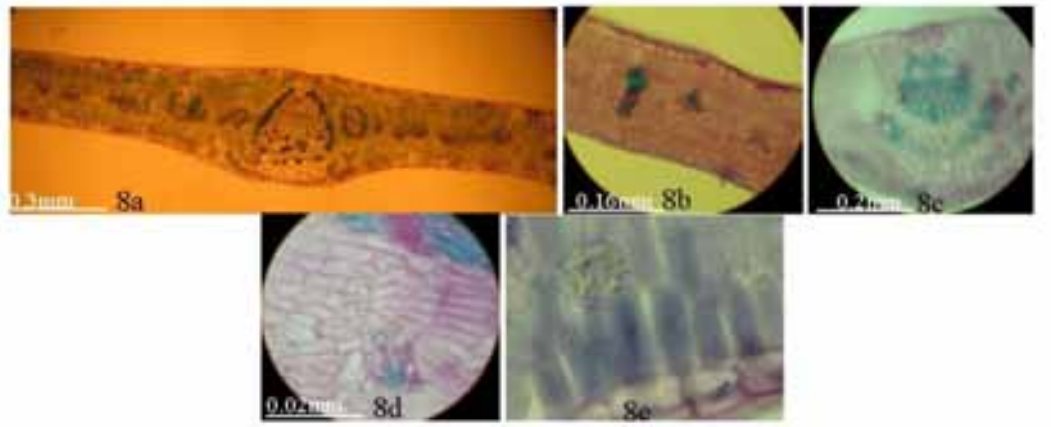

Fig. 8. *Salix wilhelmsiana*: (a-b) the lamina with palisade parenchyma in both surfaces without hypodermis layer in lower surface; (c) the midrib; (d-e) the crystals.

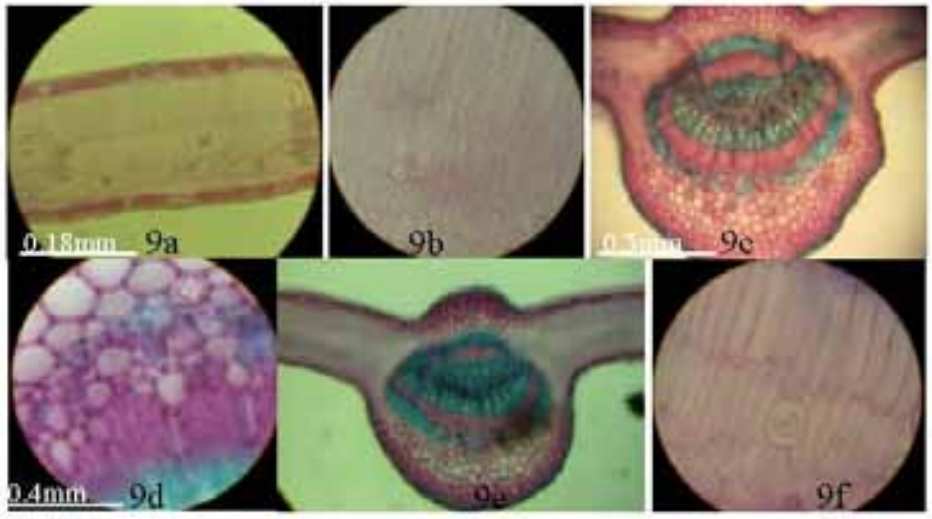

Fig. 9. *Salix zygostemon*: (a-b) the lamina with palisade parenchyma in upper surface and spongy parenchyma in lower surface; (c-d) the midrib; (e-f) the crystals.

Mesophyll. The structure of palisade and spongy tissue is important in the genus. Here the type of lamina for the species is discussed (Diane *et al*., 2003):

1. Bifacial type (B): Palisadic parenchyma is 2-layerd, and the rest of the region is filled with spongy parenchyma. This type is present in *S. aegyptiaca*, *S. caprea*, *S. cinerea*, *S. elbursensis*, and *S. zygostemon* (Figs. 1a, 2a, 3a, 4a, 9a & b)
2. Isobilateral type (two layered: I2): This type contains 2- layered palisade parenchyma in both surfaces. This type was observed in *S. euxina*, *S. issatissensis*, *S. triandra*, and *S. wilhelmsiana* (Figs. 5a, 6a, 7a, & 8a).

Shape of the upper palisade parenchyma cell walls is significant character: It is straight in *S. aegyptiaca, S. cinerea*, *S. issatissensis*, *S. zygostemon*, and *S. wilhelmsiana*; crinkled in *S. caprea* (Fig. 2a), *S. elbursensis* (Fig. 4b); alternatively more or less straight and undulate in *S. euxina* and *S. triandra.*

Spongy parenchyma cells are 2-3 layered, small and irregularly undulated with air-spaces in *S. aegyptiaca*, *S. caprea* and *S. cinerea*, but those are 3-layered, small and undulated in *S. elbursensis*.

Parenchyma ratios. The ratio of the upper palisade parenchyma thickness to the rest of mesophyll was studied. The result among the species with bifacial type lamina showed that *S. aegyptiaca* had highest ratio and *S. elbursensis* had the lowest one and between leaves with isobilateral type, *S. euxina* showed the highest ratio and *S. wilhelmsiana* had the lowest one.

Crystals. Cluster crystals (druses) are the most common type in the studied species, which are exhibited in both lamina and midrib (Figs. 1f, 3a, 4h, 5e, 7g, 8e, & 9e).

Prismatic (Figs. 1g, 3g, 4h, 7h , & 9f) and sand crystals (Figs. 3f, 4i, 6e, 7f, & 9f) with various shapes and sizes, mostly situated around the vascular bundles in leaves.

Midrib

Midrib thickness. The Midrib thickness was measured with an average thickness of 0.36 to 1 mm. *S. elbursensis* and *S. wilhelmsiana* had the thinnest and *S. cinerea* and *S. aegyptiaca* had the thickest ones. The rest species had an average thickness between 0.5 to 0.8 mm.

Cortex. Upper cortex thickness also showed some differences among the species, it had a range between 0.06 to 0.24 mm. Lower cortex thickness was from 0.1 to 0.24 mm. Annular collenchymas with uniformly thickened cell walls occurred close to the epidermis. Upper annular collenchymas were one- layered in *S. elbursensis* and *S. wilhelmsiana* to 4-5 layers in *S. issatissensis* and *S. aegyptiaca*. This was mostly 2-4 layered close to the lower epidermis except in *S. elbursensis* and *S. wilhelmsiana* that were 1-2 layered. Vascular bundles were surrounded mostly by orbicular and rarely irregular parenchyma cells that are 2-6 layers in upper cortex and 2-8 layers in lower cortex. Sclerenchymatous cells were found around the vascular system, which made a continual sheath in upper surface and are discontinued in lower surface. Sclerenchymatous cells made thicker sheath close to the lower cortex than upper one.

Vascular system. This is inserted in the central part and is singular in *S. cinerea*, *S. triandra*, *S. wilhelmsiana*, and *S. issatissensis*, but usually two bundles are found in *S. aegyptiaca*, *S. caprea*, *S. elbursensis*, *S. euxina*, and *S. zygostemon*.

DISCUSSION

The studied species were selected from two of the three subgenera according to Maassoumi (2009): *S. euxina*, *S. issatissensis*, and *S. triandra* belong to the subgenus *Salix* and *S. aegyptiaca*, *S. caprea*, *S. cinerea*, *S. elbursensis*, *S. wilhelmsiana* belonging to subgenus *Vetrix*.

S. euxina was described by Belyaeva (2009). The species was previously reported as *S. fragilis* belong to subgen. *Salix*, sect. *Salix* by Maassoumi (2009), while according to Belyaeva (2009) it is now called *S. euxina* and *S. fragilis* is the name of the hybrid *S. alba* × *S. euxina* (syn.: *S.* × *rubens*).

S. triandra belongs to Sect. *Triandrae*; *S. issatissensis*, *S. euxina* to Sect. *Salix*; *S. aegyptiaca*, *S. caprea*, and *S. cinerea* to Sect. *Cinerella*; *S. elbursensis* to Sect. *Helix*, and *S. wilhemsiana* to Sect. *Cheilophilae***.** *S. zygostemon* is an intersectional hybrid of *S. cinerea* and *S. elbursensis* (Maassoumi 2009).

Size and shape of the leaves are of high variability in the genus *Salix*. According to the anatomical data, the genus *Salix* can be divided into two types based on presence or absence of hypodermis layer in lamina: The first type (Fig. 10) with hypodermis layer including *S. euxina*, *S. issatissensis*, *S. triandra*. Metcalfe & Chalk (1957) reported existence of hypoderm within the lower epidermis in *S. alba*, *S. babylonica* L., and *S. pentandra* L. The species are all categorized in subgen. *Salix* (Maassoumi 2009). We found the second type (Fig. 10) without hypodermis layer including *S. aegyptiaca*, *S. caprea*, *S. cinerea*, *S. wihlelmsiana* and *S. elbursensis* that are all in subgenus *Vetrix* (Maassoumi 2009). Skvortsov (1999) also suggested that a hypodermis is lacking in *Salix* subg. *Vetrix*. In the studied species belong to subgen. *Salix,* leaf lamina was isobilateral and those belonging to subgen *Vetrix* leaf lamina was bifacial except in *S. wilhelmsiana* that was isobilateral.

S. aegyptiaca, *S. caprea*, *S. cinerea* belonging to sect. *Cinerella* had some especial features that are not observed in the other sections: The outline of the transverse sections exhibited round U-shape, however in other sections, midrib with less height has expanded shape. Stomata type was hypostomatic (in sect. *Cinerella*), however, in the other sections the stomata type was hypoamphistomatic. Palisade parenchyma ratio in *S. aegyptiaca*, *S. caprea*, *S. cinerea* belonging to sect. *Cinerella* was more than two; it means that the upper palisade parenchyma cells are larger than other lamina parenchyma cells in comparison with other sections.

In many plant species calcium crystals are commonly formed under ordinary conditions (Arnott & Pautard 1970). Their type and location are often used in plant taxonomic classification (Solereder 1908; Hsieh & Huang 1974; Genua & Hillson 1985). The crystals may occur in different plant organs and in various shapes, e.g. druses, prismatic crystals, raphides, styloides, and crystal sands. Solitary and clustered crystals were reported in *Salicaceae* by Metcalfe & Chalk (1957).

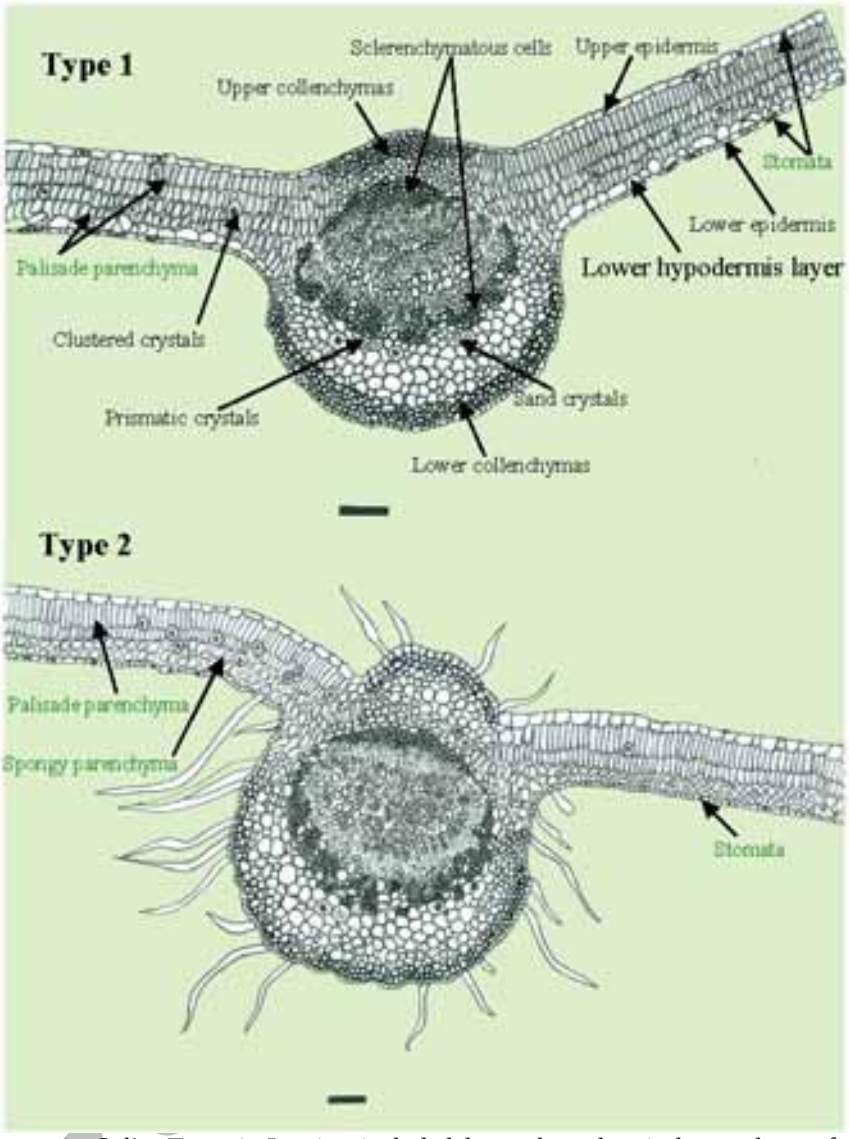

Fig. 10. Foliar types in *Salix*. Type 1: Lamina included lower hypodermis layer; drawn from *S. issatissensis* (subgen. *Salix*), scale bar = 0.14 mm. Type 2: Lamina without lower hypodermis layer; drown from *S. cinerea* (subgen. *Vetrix*), scale bar = 0.12 mm.

The delimitation of the closely related species of *S. aegyptiaca*, *S. caprea* and *S. cinerea* are difficult (Maassoumi 2009), but they can be distinguished on the basis of some anatomical characters. Palisade parenchyma cells in *S. aegyptiaca* and *S. cinerea* have straight walls whereas those are crinkled in *S. caprea*. Semi-clustered crystals, extremely expanded crystals, were observed only in *S. aegyptiaca* and not in *S. caprea*, and *S. cinerea*, or any other species studied here.

Another interesting result was about the studied hybrid. *S. zygostemon* is inter-specific hybrid of *S. cinerea* and *S. elbursensis* (Massoumi 2009). The anatomical data on Tables 2, and 3 show that *S. zygostemon* mostly has midrib anatomical features close to *S. cinerea* and lamina anatomical features close to *S. elbursensis*.

Acknowledgment

We are deeply grateful to the administration and staff of the Research Institute of Forests and Rangelands.

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