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LEAF ANATOMICAL STUDY OF *SOLANUM* SPECIES (SOLANACEAE) IN IRAN

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Solanum (Solanaceae) comprises cultivated and wild plants with 1400 species in the world and 14 species in Iran. *Solanum* is a taxonomically complex genus due to morphological similarities, phenotypic plasticity and hybridisation. Limited studies were done on anatomical features of this important genus. In this project, 10 native and exotic species of *Solanum* in Iran belonging to two subgenera were examined anatomically. Leaf mesophyll and midrib and indumentum were analysed using light microscope. Hand-made cross section method and Toluidine blue as colouring agent were used. Characters as length and width of main vascular bundle, thickness of collenchyma, trichome density, thickness of parenchyma strand, thickness of lamina and length and shape of midrib were diagnostic features among species studied. In UPGMA tree and PCA ordination, species of two subgenera were separated from each other. Results of this study confirmed the taxonomic importance of anatomical characters in *Solanum* species studied.

Key words: mesophyll, midrib, subgen. *Leptostemonum*, subgen. *Solanum.*

INTRODUCTION

Solanum L., with 1400 species in the world, is the largest genus of the Solanaceae family (Frodin 2004, Särkinen *et al.* 2013). South America is the main centre of *Solanum* diversity. This genus is widely distributed worldwide which occupies different habitats and altitudes from the wettest forests to driest deserts and from the sea level to over 4,500 m altitude (McClelland *et al.* 2020). This genus comprises herbs, shrubs and small trees with simple or pinnate compound leaves, racemose, paniculate or umbellate inflorescence, bisexual or andromonoecious flowers, and berry fruits (Boissier 1879, Schonebeck-Temesy 1972).

Solanum is considered of economic and medicinal importance. The cultivated taxa as potato (*S. tuberosum* L.), tomato (*S. lycopersicum* L.), and eggplant (*S. melongena* L.) are among the edible vegetables and fruits in the world (Muthoni *et al.* 2012). Moreover, different species of *Solanum* contain alkaloids, flavonoids, solasodine, and tannins, which can be used as fever-reducing agents, pain relievers, diarrhoea, softener, and anti-asthma (Eskandari *et al.* 2019, Pereira *et al.* 2014, Rajathi *et al.* 2015, Ravi *et al.* 2009).

Solanum shows a complex taxonomic situation. Morphological similarities, hybridisation followed by introgression, and phenotypic plasticity make species delimitation difficult (Bello *et al.* 2013, Levin *et al.* 2005, Spooner and van den Berg 1992). Linnaeus (1753) was the first who divided *Solanum* into two groups of *Spinosa* and *Inermis*. D'Arcy (1972) divided *Solanum* into different subgenera, sections, and series. Hunziker (2000) has modified D'Arcy's classification. He described and explained sections. Levin *et al.* (2006) and Stern *et al.* (2010) used the molecular data to taxonomically rearrange *Solanum* species above the sectional level.

Members of this genus in Iran are classified into two subgenera: *Solanum* and *Leptostemonum* and seven sections, including *Petota*, *Lycopersicon*, *Holophylla*, *Solanum*, *Dulcamara*, *Acanthophora* and *Melongena*. Subgenus *Leptostemonum* is the largest monophyletic group within *Solanum* containing nearly half of *Solanum* species (Stern *et al.* 2011). Aubriot *et al.* (2016) stated that about 240 members of this subgenus are limited to Old World tropics. This group has significant centre of diversity in Australia (Symon 1981).

Different authors recorded various numbers of *Solanum* for Iran. Schonebeck-Temesy (1972) described 12 species of *Solanum* in Iran while Khatamsaz (1998) identified 10 species. Furthermore, during the years, some new taxa of *Solanum* were recorded from different parts of Iran as *S. elaeagnifolium* (Mozaffarian 1994), *S. sisymbriifolium* (Eslami and Naqinezhad 2011) and *S. viarum* (Eskandari and Abdi Fouladkolaei 2020). Recent studies confirmed 14 species of *Solanum* in Iran out of which six species are native in Iran (Eskandari 2020).

Literature on leaf anatomical studies of *Solanum* species is not adequate. Rogers and Ogg (1981) studied *S. nigrum* complex morphologically and anatomically. They reported the anomocytic and anisocytic stomata in species studied with the larger stomata in *S. nigrum*. Sanghvi *et al.* (2011) studied the leaf anatomy of *S. pseudocapsicum*. They reported uni-layer palisade and four layers of spongy mesophyll with uniseriate trichomes on the epidermis surface. Kristić *et al.* (2002) considered leaf anatomical characters in different populations of *S. nigrum*. Characters as palisade and spongy mesophyll thickness, size of mesophyll cells, number of stomata and number of hairs showed differences in different populations. Alves *et al.* (2007) reported different types of trichomes in the leaf surface of *S. cernuum*. Studying the leaf anatomy in *S. pseudocapsicum* showed dorsiventral mesophyll with collateral vascular bundles. Furthermore, uniseriate trichomes were observed on the leaf surface (Sanghvi *et al.* 2011).

As *Solanum* is an important genus taxonomically and economically and as there is no anatomical study on *Solanum* species in Iran, the objectives of this study are to investigate the leaf anatomical characters to identify different species and determine if anatomical differences corroborate subgenera and sectional levels within this genus.

Table 1
Voucher details of *Solanum* species studied (asterisks show native species)

Subgenus	Section	Taxon	Locality and voucher no.
Solanum	*Dulcamara*	* *S. dulcamara* L.	Golestan, 5 km of Kalaleh to Dahaneh. IRAN-74539
			Tehran, Darakeh. IRAN-74782
			Alborz, Taleghan. IRAN-74784
			Chaharmahal and Bakhtiari, Ardal, Sar Tang-e Mahmud village. IRAN-40479
			Mazandaran, Savadkuh, Veresk village. IRAN-74788
		* *S. kieseritzkii* C. A. Mey	Golestan, Gorgan, Shamushak forest. IRAN-74537
	Lycopersicon	*S. lycopersicum* L.	Mazandaran, Amol to Mahmudabad, Caspian forest seed centre. IRAN-74778
	Solanum	* *S. nigrum* L.	Markazi, Delijan. ALUH-38770
			Mazandaran, 4 km to Ramsar. ALUH-38761
		* *S. villosum* Mill.	Hormozgan, Bashagard, Jakdan village. IRAN-40451
			Gilan, Amlash, Halu Dasht. IRAN-75781
	Holophylla	*S. pseudocapsicum* L.	Tehran, Evin. IRAN-74785
Leptostemonum	*Melongena*	*S. elaeagnifolium* Cav.	Tehran, Bagher shahr, Darsunabad village. IRAN-74876
		* *S. incanum* L.	Sistan and Baluchestan, Chabahar, Konarak, Pozm village. IRAN-74051
		S. sisymbriifolium Lam.	Gilan, Rasht, Saravan Forest, near Saravan to Fuman road. IRAN-75788
		* *S. surattense* Burm. f.	Sistan and Baluchestan, Iranshahr to Chabahar, Sarbaz bridge. IRAN-74063

MATERIALS AND METHODS

Sixteen accessions of 10 species from five sections of *Solanum* were studied. Fresh plants were gathered from nature during 2019–2020. For some samples, the herbarium specimens were used. Different taxonomic literature as Flora Iranica (Schonebeck-Temesy 1972) and Flora Orientalis (Boissier 1879) were used to identify the specimens. Vouchers are deposited at Herbarium of Alzahra University (ALUH) and Herbarium of Iranian Research Institute of Plant Protection, Tehran, Iran (IRAN) (Table 1).

Leaf samples were selected from the middle part of stem and fixed in FAA solution for 7–10 days. For the anatomical study, the handmade sections were done using razor from the middle parts of mature intact leaves. Toluidine blue was used as the colouring agent. Cross sections were observed with the light microscope (Olympus DP12). In total, 12 quantitative and 4 qualitative characters were used for studying (Table 2).

For the multivariate statistical analysis, the mean of quantitative characteristics was used, while the qualitative ones were coded as binary/multistate characteristics. For each population, 3–5 leaf samples were examined. For each characteristic, five measurements were performed and mean and standard deviation values were obtained for each one. Standardised variables were used for the multivariate statistical analysis. Analyses were done using PAST ver. 3.23 software (Hammer *et al*. 2001). To determine similarities, cluster analysis and ordination by the principal component analysis were done.

Table 2
Quantitative and qualitative leaf anatomical characters

1. Upper epidermis thickness	9. Width of midrib
2. Lower epidermis thickness	10. Thickness of collenchyma in ventral surface
3. Thickness of parenchyma strand in ventral surface of midrib	11. Thickness of collenchyma in dorsal surface
4. Thickness of parenchyma strand in dorsal surface of midrib	12. Number of palisade parenchyma layer
5. Thickness of lamina	13. Trichome type (glandular 1, non-glandular 2, both types 3)
6. Length of main vascular bundle	14. Trichome density (low 1, moderate 2, high 3)
7. Width of main vascular bundle	15. Shape of midrib cross section (triangular 1, circular 2, ovate 3, – except those 4)
8. Length of midrib	16. Mesophyll type (dorsiventral 1, isobilateral 2)

RESULTS

The anatomical characteristics are summarised in Table 3. All studied species showed a uniseriate epidermis in both surfaces composed of mostly rectangular to polygonal shaped cells (Fig. 1). *Solanum* species showed different thicknesses of upper and lower epidermis. The highest and the lowest thickness of upper epidermis were seen in *S. sisymbriifolium* and Sar Tang-e Mahmud population of *S. dulcamara*, respectively, while the highest and the lowest thickness of lower epidermis were observed in Jakdan village population of *S. villosum* and Golestan population of *S. villosum*, respectively.

Glandular and non-glandular types of trichomes were observed on the leaf surfaces of species/populations studied. In the epidermis layer of *S. dulcamara*, unicellular and bicellular short non-glandular trichomes, multicellular simple and hooked non-glandular trichomes in different sizes and glandular trichomes with unicellular stalk and multicellular head were seen (Figs 2A–F). Trichome density in populations of *S. dulcamara* was different. Golestan population showed a high density of trichomes, but Veresk, Taleghan, and Darakeh populations showed moderate trichome density. In the Sar Tang-e Mahmud population, trichome density was very low. In *S. pseudocapsicum* and *S. lyco-*

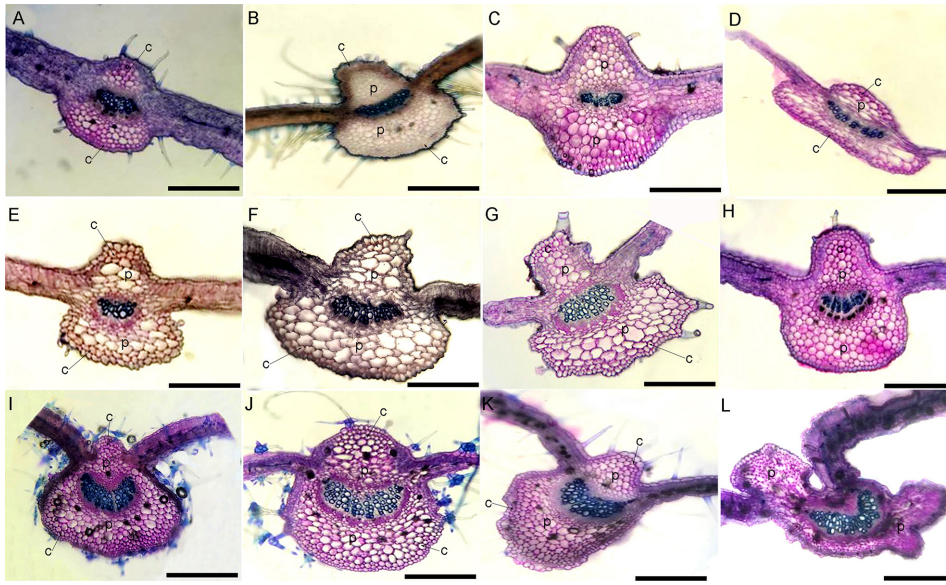

Fig. 1. Midrib cross sections in species studied. A = *S. dulcamara* (Darakeh population), B = *S. dulcamara* (Golestan population), C = S. *kieseritzkii*, D = *S. lycopersicum*, E = *S. nigrum* (Mazandaran population), F = *S. villosum* (Hormozgan population), G = *S. villosum* (Gilan population), H = *S. pseudocapsicum*, I = *S. elaeagnifolium*, J = *S. incanum*, K = *S. sisymbriifolium*, L = *S. surattense* (c = collenchyma, p = parenchyma) (scale bar: 200 μm)

Table 3
Summary of measured and evaluated characters in species/populations studied (character no. based on Table 2, measurements are in μm)

Taxon	1	2	3	4	5	6	7
S. dulcamara (IRAN-74539)	7.85±0.74	5.49±2.23	10.6.78±0.28	114.13±0.30	64.95±4.01	51.96±1.72	144.53±9.61
S. dulcamara (IRAN-74782)	9.86±0.75	9.56±1.20	57.24±0.27	49.30±6.18	124.42±4.55	51.33±0.81	107.91±5.41
S. dulcamara (IRAN-74784)	8.59±3.06	7.68±0.54	43.18±0.41	38.88±3.07	89.03±3.99	93.31±3.20	141.17±5.18
S. dulcamara (IRAN-40479)	6.07±2.80	7.99±2.60	45.48±1.30	29.15±1.28	75.78±1.88	65.48±2.57	154.69±14.16
S. dulcamara (IRAN - 74788)	14.18±2.91	8.89±1.17	30.71±2.07	49.63±1.20	110.02±10.17	75.83±9.96	200.14±5.29
S. kieseritzkii	13.11±3.48	17.58±4.87	182.97±3.38	183±7.34	152.39±14.15	57.89±4.67	152.65±3.21
S. lycopersicum	11.66±3.75	8.33±3.37	59.33±1.42	39.66±5.83	48.70±6.94	47.56±1.72	194.14±32.30
S. nigrum (ALUH-38770)	9.73±0.73	11.43±3.83	51.48±0.75	100.37±4.84	81.51±4.76	80.53±1.58	122.09±16.43
S. nigrum (ALUH-38761)	12.74±4.34	15.87±3.29	67.75±13.24	60.80±14.05	65±3.53	69.71±0.59	108.59±2.99
S. villosum (IRAN-40451)	17.15±5.55	18.07±5.16	125.64±3.30	100.177±13.15	119.72±26.95	81.51±7.4	196.77±7.9
S. villosum (IRAN-75781)	10.32±2.37	11.15±1.31	114.38±0.54	104.99±8.13	71.44±1.93	120.78±7.85	195.81±6.51
S. pseudocapsicum	11.81±2.38	9.07±4.05	48.80±5	127.41±2.17	87.91±5.41	82.68±4.76	157.17±2.79
S. elaeagnifolium	10.62±1.6	7.36±1.12	42.69±4.7	96.01±11.53	116.48±8.32	166.48±11.29	270.26±18.19
S. incanum	13.19±3.61	11.75±2.43	127.54±3.35	127.24±6.42	82.97±9.74	158.68±9.87	312.50±17.27
S. sisymbriifolium	17.94±3.79	11.62±4.08	66.58±1.23	85.32±5.4	93.97±3.37	135.78±1.83	220.67±11.69
S. surattense	11.09±5.48	6.89±2.86	259.61±7.14	112.50±16.32	139.96±31.31	124.22±12.95	193.15±36.53

Table 3 (continued)

Taxon	8	9	10	11	12	13	14	15	16
S. dulcamara (IRAN-74539)	348.81±1.17	305.66±2.12	27.95±0.45	13.34±3.45	1	3	3	1	1
S. dulcamara (IRAN-74782)	266.48±3.74	262.56±14.86	10.26±0.49	10.09±1.43	1	3	2	2	1
S. dulcamara (IRAN-74784)	274.75±0.53	221.80±13.21	13.50±1.31	8.98±0.49	1	2	2	1	1
S. dulcamara (IRAN-40479)	247.85±0.66	313.19±3.21	17.75±1.92	19.13±2.63	1	2	1	1	1
S. dulcamara (IRAN - 74788)	304.72±5.97	374.23±7.41	27.79±1.25	10.41±2.05	1	3	2	1	1
S. kieseritzkii	504.68±10.06	504.38±41.25	0	0	1	2	1	3	1
S. lycopersicum	224.72±5.94	547.87±7.01	16.15±5.60	10.07±1.28	1	3	1	1	1
S. nigrum (ALUH-38770)	277.97±8	361.02±12.81	0	0	1	3	2	1	1
S. nigrum (ALUH-38761)	281.59±2.65	277.65±13.69	40.94±3.61	18.76±5.20	1	2	1	1	1
S. villosum (IRAN-40451)	436.58±6.57	489.09±25.81	24.76±1.5	12.23±2.74	1	1	1	1	1
S. villosum (IRAN-75781)	439.17±5.27	384.48±35.08	27.42±4.67	16.26±0.72	1	2	1	1	1
S. pseudocapsicum	467.48±10.41	411.67±11.38	63.54±3.44	0	1	3	1	1	1
S. elaeagnifolium	588.34±3.26	671.79±2.5	83.16±7.35	65.98±5.7	4	3	3	1	2
S. incanum	600.46±5.93	513.44±17.55	69.83±4.55	45.49±6.54	1	3	3	1	1
S. sisymbriifolium	551.60±18.55	396.78±28.03	83.55±8.49	94.29±10.04	2	3	3	3	1
S. surattense	785.19±37.46	322.09±19.74	0	0	5	2	1	4	2

persicum, low numbers of glandular and non-glandular trichomes were observed. Both species had short-stalked glandular trichomes with spherical and multicellular head (Figs 2H, 2L), and hooked multicellular non-glandular trichomes (Figs 2I, 2K). In *S. lycopersicum*, spine-like, unicellular, and bicellular non-glandular trichomes were also seen (Figs 2J, 2M). Branched multicellular non-glandular trichomes were only observed in *S. pseudocapsicum* (Fig. 2G). Different populations of *S. nigrum* had glandular trichomes with multicellular stalk and unicellular head and uni- to multicellular non-glandular ones with low to moderate density (Figs 2N–P).

S. villosum and *S. kieseritzkii* had a low density of bicellular non-glandular trichomes (Figs 3B, 3C). *S. villosum* also showed little numbers of sessile glandular trichomes (Fig. 3A).

S. elaeagnifolium showed a high density of unicellular short non-glandular, tri- to multi-radiate stellate non-glandular trichomes (Figs 3D–F) and some glandular trichomes with multicellular head (Fig. 3G). In *S. incanum*, a high density of tri- to multi-radiate stellate non-glandular trichomes was observed on both leaf surfaces (Fig. 3H). Besides, a very low number of short-stalked glandular trichomes with multicellular head can be seen on the ventral epidermis (Fig. 3I). *S. sisymbriifolium* showed different shapes of trichomes with moderate density. Uni-, bi- to multicellular long trichomes, tri- to multi-radiate stellate trichomes of non-glandular type, and trichomes with uni- to multicellular stalk and uni- to multicellular head of glandular type occurred

on the leaves of *S. sisymbriifolium* (Figs 3J–M). *S. surattense* showed a very low density of tri-radiate stellate non-glandular trichomes (Fig. 3N).

The mesophyll of *Solanum* species studied was dorsiventral and isobilateral with different layers of palisade parenchyma. In *S. elaeagnifolium* and *S. surattense*, isobilateral mesophyll with 4 and 5 layers of palisade parenchyma was seen, respectively (Figs 4G, 4J), while in other species, dorsiventral mesophyll with one layer of palisade parenchyma were observed (Figs 4A-F, 4H, 4K). *S. sisymbriifolium* had bi-layered palisade parenchyma (Fig. 4I).

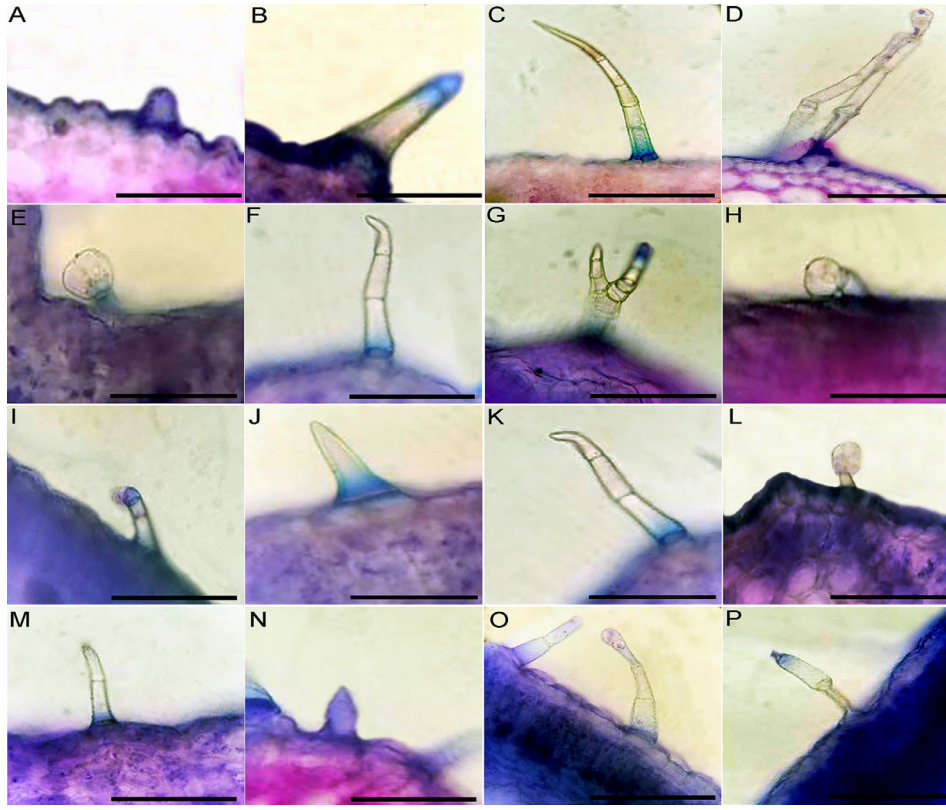

Fig. 2. Different types of trichome in species studied. A–F = *S. dulcamara* (A = unicellular short non-glandular trichome, B = bicellular non-glandular trichome, C = multicellular non-glandular trichome, D = long-stalked glandular trichome, E = short-stalked glandular trichomes with multicellular head, F = hooked non-glandular trichome), G–I = *S. pseudocapsicum* (G = branched multicellular non-glandular trichome, H = short-stalked glandular trichomes, I = hooked multicellular non-glandular trichome), J–M = *S. lycopersicum* (J = spine-like non-glandular trichome, K = hooked multicellular non-glandular trichome, L = short-stalked glandular trichome, M = bicellular non-glandular trichome), N–P = *S. nigrum* (N = unicellular non-glandular trichome, O = glandular trichome with multicellular stalk and bicellular non-glandular trichome, P = glandular trichome) (scale bar: 250 μm)

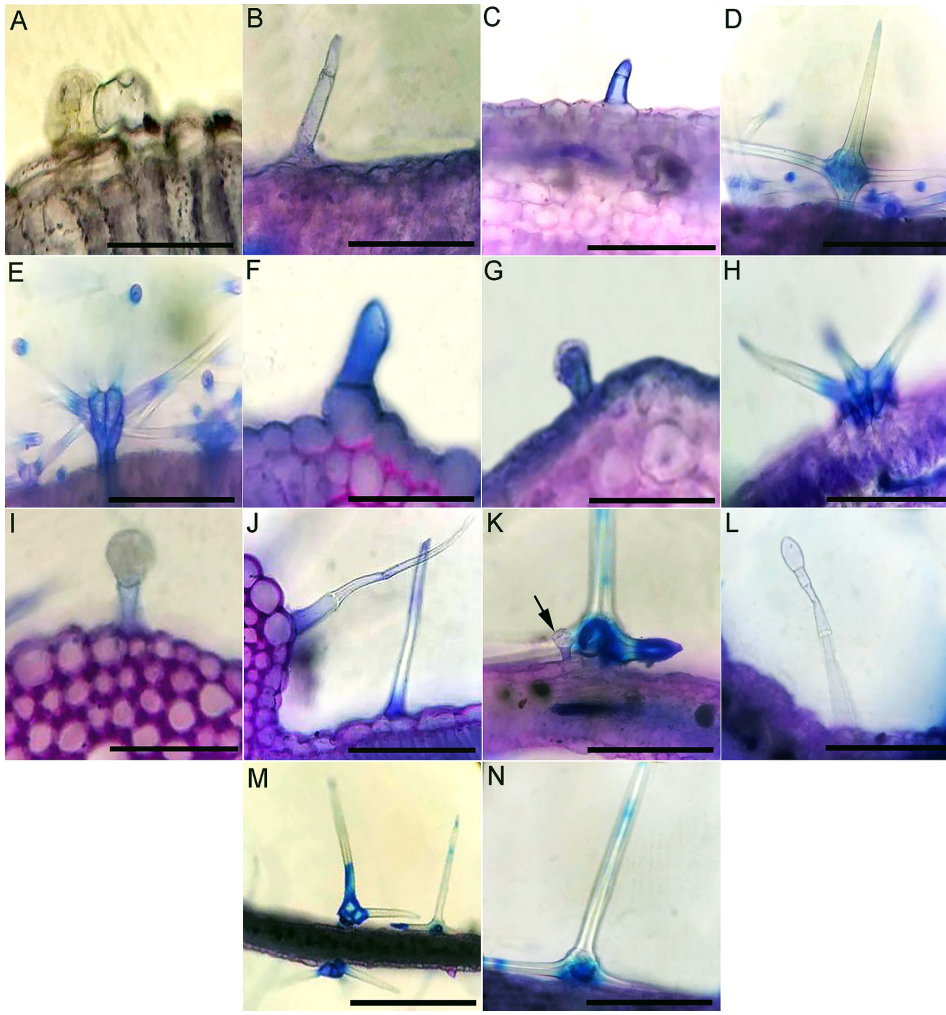

Fig. 3. Different types of trichome in species studied. A–B = *S. villosum* (A = sessile glandular trichome, B = bicellular non-glandular trichome), C = *S. kieseritzkii* (short bicellular non-glandular trichome), D–G = *S. elaeagnifolium* (D = tri-radiate stellate non-glandular trichome, E = multi-radiate stellate non-glandular trichome, F = unicellular short non-glandular trichome, G = glandular trichome with multicellular head), H–I = *S. incanum* (H = multi-radiate stellate non-glandular trichome, I = short-stalked glandular trichomes with multicellular head), J–M = *S. sisymbriifolium* (J = uni and multicellular non-glandular trichome, K = tri-radiate stellate non-glandular trichome and glandular trichome with multicellular head, L = multicellular long stalk of glandular trichome, M = tri- and multi-radiate stellate non-glandular trichomes), N = *S. surattense* (tri-radiate stellate non-glandular trichome) (scale bar = 250 μm)

Thickness of lamina varied from 152.39 μm in *S. kieseritzkii* to 48.7 μm in *S. lycopersicum*. Calcium oxalate crystals were only detected in mesophyll and main vein of *S. dulcamara*, *S. pseudocapsicum*, *S. elaeagnifolium*, *S. incanum*, *S. sisymbriifolium* and *S. surattense* (Table 3).

Different shapes of midrib outline were observed in the species studied: the triangular shape was the most common among the taxa (Figs 4B, 4D–J), circular shape in Darakeh population of *S. dulcamara* (Fig. 1A), and ovate shape in *S. kieseritzkii* and *S. sisymbriifolium* (Figs 1C, 1K). In *S. surattense* shape of midrib outline was completely different from other populations/species studied (Fig. 1L). Except for *S. kieseritzkii*, *S. surattense* and Delijan population of *S. nigrum* (Figs 1C, 1L), other species showed collenchyma in abaxial and adaxial surfaces of midrib. In *S. pseudocapsicum* there is no collenchyma on the abaxial surface (Fig. 1H). *S. sisymbriifolium* had the thickest collenchyma of both surfaces (Fig. 1K).

Parenchyma was the main tissue of midvein showing variations in shape, size and, thickness. The highest thickness of parenchyma on the main vein belonged to *S. surattense* adaxially and *S. kieseritzkii* abaxially (Figs 1C, 1L), whereas the lowest thickness belonged to different populations of *S. dulcamara* (Figs 1A–B, Table 3). The main vein of all examined species consisted of a single vascular bundle. In all studied taxa, bicollateral vascular bundle was observed (Fig. 1). In *S. surattense*, the midrib length was the highest (785.19 μm) and in *S. lycopersicum* (224.72 μm) was the lowest.

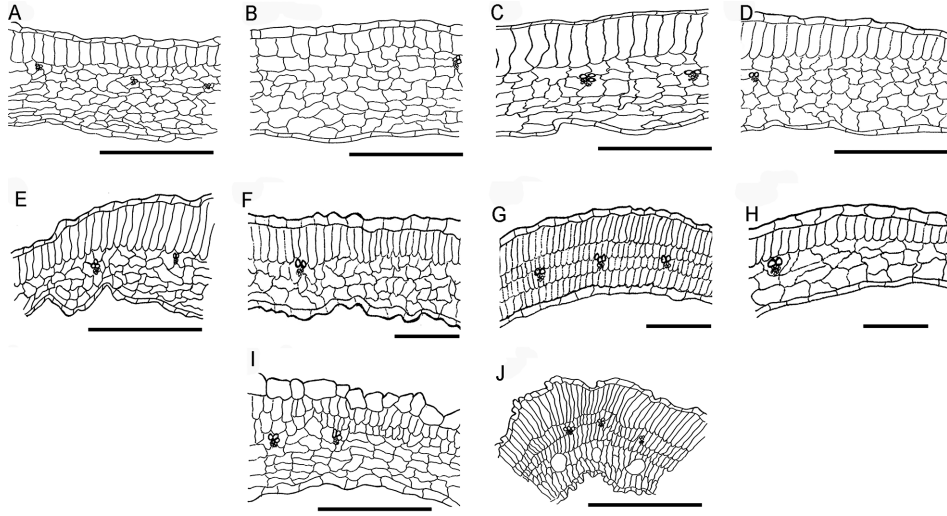

Fig. 4. Schematic drawing of lamina cross section in species studied. A = *S. dulcamara*, B = *S. kieseritzkii*, C = *S. lycopersicum*, D = *S. nigrum*, E = *S. villosum*, F = *S. pseudocapsicum*, G = *S. elaeagnifolium*, H = *S. incanum*, I = *S. sisymbriifolium*, J = *S. surattense* (scale bar in A–E, I–J = 200 μm; scale bar in F–H = 250 μm)

UPGMA tree using Euclidian distance showed two main clusters. Members of subgen. *Leptostemonum* were placed in the first main cluster and members of subgen. *Solanum* were grouped in the second one. *S. kieseritzkii* from subgen. *Solanum* was placed in a separate sub-cluster. In this analysis, *S. villosum* population were nested near *S. pseudocapsicum*. These two species showed similarities in characteristics as trichome density, mesophyll type, the shape of midrib cross section, and type of vascular bundle (Fig. 5).

Principle Component Analysis (PCA) showed that first three axes explained 78.67% of the total variation. In the first axis with 39.83% of the variation, length and width of main vascular bundle and thickness of collenchyma in dorsal and ventral surfaces had the highest correlation (> 0.7). Features as the thickness of parenchyma strand in ventral surfaces of midrib and shape of midrib cross section were responsible for the second axis with 29.13% of the variation. In the third axis with 9.71% of variation, lower epidermis thickness showed the highest correlation.

According to PCA ordination, *S. kieseritzkii* was placed far from other species of subgen. *Solanum*. Grouping and the relationship of other species were similar to the UPGMA tree (Fig. 6).

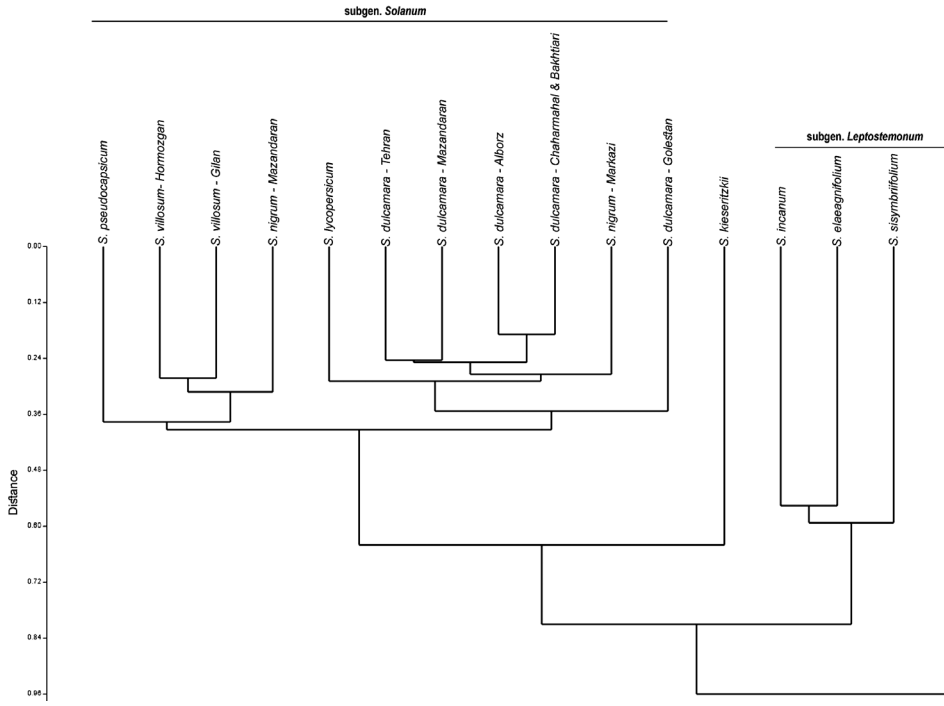

Fig. 5. UPGMA tree of studied species based on anatomical characters

DISCUSSION

Leaf anatomical and epidermal characters provided useful information for taxonomy and delimitation of *Solanum* species (de Rojas 2007, Nurit-Silva *et al.* 2012, Roe 1972, Sampaio *et al.* 2014). Previous studies reported single layer of the epidermis in both surfaces of different *Solanum* species (Burrows *et al.* 2013, Kristić *et al.* 2002, Sanghvi *et al.* 2011). Our results were in agreement with them.

Solanum had different indument on the leaf surface. The types of glandular and non-glandular trichomes can be observed on abaxial and adaxial surfaces of *Solanum* leaves. Different functions were documented for trichomes as defense barriers against the insect herbivores, cooling the leaf and reducing transpiration and leaf wettability (Brewer and Nuñez 2007, Kariyat *et al.* 2017, Skelton *et al.* 2012). The morphology of the trichome in *Solanum* was considered taxonomically important at infrageneric and interspecific levels (Adedeji *et al.* 2007, Nurit-Silva *et al.* 2012, Sampaio *et al.* 2014). Despite similar types of trichomes observed in *Solanum* species studied, stellate trichomes were only seen in taxa of subgen. *Leptostemonum*, which in concordance with previous results (Burrows *et al.* 2013, Christodoulakis *et al.* 2009, Kristić *et al.* 2002).

Metcalf and Chalk (1950) considered dorsiventral leaves as the usual type in Solanaceae. The type of mesophyll was considered a diagnostic character in taxa studied. The mesophyll types found in studied species were dorsiventral and isobilateral that were in accordance with previous literatures (Burrows *et al.* 2013, De Micco *et al.* 2014, Kristić *et al.* 2002, Matias *et al.* 2016, Sanghvi *et al.* 2011).

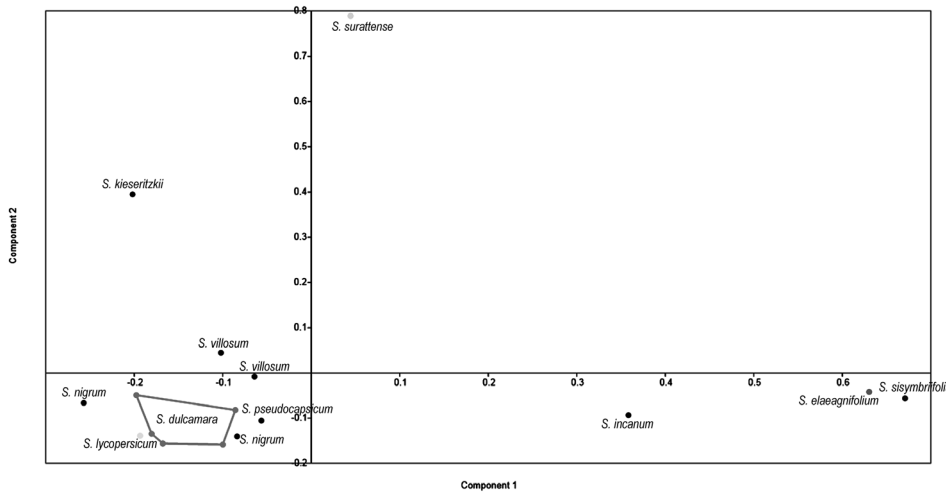

Fig. 6. PCA ordination of studied species based on anatomical characters

The number of palisade parenchyma layers was considered significant in some *Solanum* species studied. Although most of analysed taxa showed only one layer of palisade parenchyma, multi-layer of palisade parenchyma was observed in *S. elaeagnifolium*, *S. surattense* and *S. sisymbriifolium*. According to Kristić *et al.* (2002), lamina of *S. nigrum* had one layer of palisade and 3-5 layers of spongy parenchyma. We found the same pattern in our studies. Sanghvi *et al.* (2011) studied the morphology and anatomy of *S. pseudocapsicum* and pointed to one layer of palisade parenchyma in leaf mesophyll which was in agreement with us. Based on Burrows *et al.* (2013), leaves of *S. elaeagnifolium* had four layers of palisade parenchyma with a dense covering of trichome, similar to our observations.

All species showed bicollateral vascular bundle in the main vein supporting previous studies (De Micco *et al.* 2014, Kristić *et al.* 2002, Matias *et al.* 2016, Sanghvi *et al.* 2011).

According to the UPGMA dendrogram, studied species were divided into two groups; species of subgen. *Leptostemonum* and species of subgen. *Solanum*. Our results were in agreement with the previous subgenus division within *Solanum* (D'Arcy 1972, 1991, Eskandari 2020, Schonebeck-Temesy 1972). Subgen. *Leptostemonum* known as spiny solanums is the largest group within *Solanum* (Stern *et al.* 2011). Morphologically, members of this subgenus had prickles and flowers with long anthers while members of subgen. *Solanum* lacked prickles and showed small ovate anthers in their flowers (Eskandari 2020, Khatamsaz 1998).

Based on the studied characters, we can say that the anatomical features are of taxonomic value in subgenera and species delimitation of *Solanum* in Iran.

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