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ARTICLE

Comparative anatomical and micromorphological study of some *Rumex* species (Polygonaceae)

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ABSTRACT *Rumex* (Polygonaceae) is a large genus of annual, biennial and perennial species in temperate regions of the world. In Iran it is represented by 23 species and some hybrids classified in three subgenera. The species identification is difficult due to the importance of fruit features in species separation despite the fact, that plants lose their flower and some other features while bearing fruits. Providing the individuals with the proper set of diagnostic features is very difficult. There are inadequate anatomical studies of *Rumex*. The present study reports the first detailed stem anatomy and epidermis micromorphology of 6 species of *Rumex* in Iran. Main aims of this study were to find the diagnostic value of the adopted features. Cross sections were made by hand and double colored. Dorsal and ventral leaf epidermises were studied by Scanning Electron Microscopy (SEM). Results of stem anatomical study showed that collateral vascular bundle is only present in *R. chalepensis* and oxalate calcium druse crystals were only absent in *R. elbrusensis*. The micro-morphological study of epidermis showed that all species studied had anisocytic stomata type, but there were differences in the epidermis and stomata cell size. Species relationships based on the results have been discussed.
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Introduction

Polygonaceae is a complex family with 59 genera and nearly 1200 species in the world (The Plant List 2013). *Rumex* L. is a large genus with 200 species, which are distributed in different habitats of temperate regions of the world (Sanchez and Kron 2008; Chase and Reveal 2009). In Iran it is represented by 23 species and some hybrids classified in three subgenera as subgenus *Acetosella*, subgenus *Acetosa* and subgenus *Rumex* from which the latter is the largest with more species (Rechinger 1968; Mozaffarian 1988).

These are annual, biennial and perennial herbs or rarely suffrutices with basal and cauline leaves. Flowers are hermaphrodite, polygamous or unisexual with 6-segments perianth and 6 stamens arranged in panicle, cyme or axile inflorescences (Rechinger 1968). Rumex species are distributed in different habitats of Iran and are of medicinal importance in folk medicine (Mozaffarian 2015).

The species identification is somehow difficult due to the importance of fruit features in species separation. From the other hand, the plants loss their flower and some other features while bearing fruits so finding individuals with the proper set of diagnostic features is difficult (Rechinger 1968). Anatomical studies in Polygonaceae are mainly focused on leaf anatomy. Leaf anatomical observation provided some diagnostic features in *Polygonum*, *Rumex*, *Persicaria*, *Fagopyrum*, *Pteropyrum* and *Rheum* (Lersten and Curtis 1992; Hameed et al. 2010; Yasmin et al. 2010 a, b; Keshavarzi et al. 2012; Soleimani et al. 2014). Stem anatomy of some *Polygonum* species illustrated that anatomical features can be valuable in species delimitation especially about similar taxa (Nazem Bokaee et al. 2015). Micromorphology of epidermis in Polygonaceae has been studied by different authors. Ronse Decraene and Akeroyd (1988) illustrated the diagnostic value of epidermis. Hong et al. (1998) focused on the tepal micromorphology and found main differences in Polygonaceae. Yasmin et al. (2010 a) studied the leaf epidermis of selected *Persicaria* species. Tahey found variations in size and shape of epidermal cells, stomata, glandular and non-glandular trichomes. They used micro-morphological features of epidermis to elucidate relationship among different taxa.

There are inadequate literatures about the internal structure of *Rumex* (Joschi 1936; Li et al. 2008; Hameed et al. 2010; Sahney and Vibhasa 2012). The present study reports the first detailed stem and epidermis anatomy of six *Rumex* species as: *R. chalepensis* Mill., *R. dentatus* L., *R. elbrusensis* Boiss., *R. conglomerates* Murray, *R. pulcher*

L., and *R. vesicarius* L. Main aims of this study were to illustrate the stem and epidermis anatomical features of the studied species and to discuss diagnostic value of the adopted features.

Materials and Methods

Six different *Rumex* species were studied from the viewpoint of their stem anatomy and epidermis structure (Table 1). Materials were gathered from nature during 2015-2017 in summer and autumn. All studied vouchers are deposited in Herbarium of Alzahra University (ALUH). For each specimen, proper replications were used. Anatomical structures of stem were studied by 4 quantitative features and 8 qualitative ones (Table 2). Anatomical structures were studied in manually sliced cross sections, after double staining with methyl green and Congo red. Cross sections were subsequently observed with an Olympus DP 12 light microscope.

Table 1. Voucher details of *Rumex* species studied (asterisk marks perennial species).

Subgenus	Species	Locality
Rumex	*R. chalepensis* *	Alborz, Karaj, 1202 ALUH
		Guilan, Bandare- Anzali, 1201 ALUH
		Khorasan Razavi, Torbat-e Heydarieh, 1206 ALUH
		Khorasan Razavi, Quchan, 1203 ALUH
		Khuzestan, Masjed Soleyman, 1209 ALUH
		Mazandaran, Amol, 1205 ALUH
		Mazandaran, Sari, 1602 ALUH
		Qom, 20 km of Qom-Kashan, Pasangan, 1207 ALUH
		Tehran, Abali, 1245 ALUH
		Tehran, Darakeh, 1208 ALUH
	*R. conglomeratus**	Guilan, Bandar-e Anzali, 1211 ALUH
		Guilan, Rudsar, 1212 ALUH
		Guilan, Sowme'eh Sara, 122 ALUH
		Mazandaran, Amol, 1217 ALUH
	R. dentatus	Tehran, Vanak, 1502 ALUH
		Khuzestan, Behbahan, 1214 ALUH
	*R. elbrusensis**	Alborz, Mardabad, 1246 ALUH
		Guilan, Rasht, 1223 ALUH
	*R. pulcher**	Alborz, Malard, 2255 ALUH
		Tehran, Taleghani Park, 1229 ALUH
Acetosella	*R. vesicarius*	South Khorasan, Ozbagu, 5241 ALUH
		Fars, Kazerun, 1200 ALUH

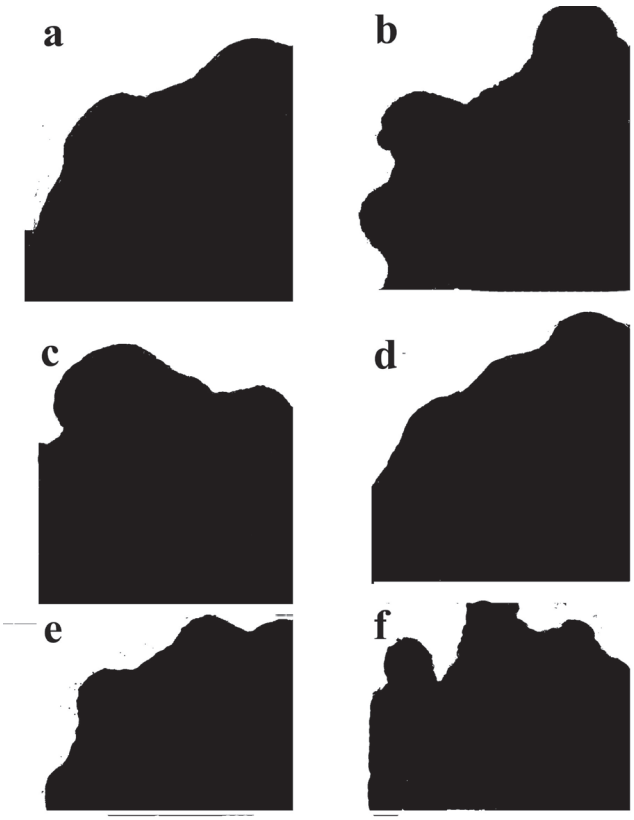

Figure 1. General shape of stem cross section in *Rumex* species studied. a) *R. chalepensis*; b) *R. conglomeratus*; c) *R. dentatus*; d) *R. elbrusensis*; e) *R. pulcher*; f) *R. vesicarius*.

The dorsal and ventral leaf epidermis of all the studied six species were examined by SEM. For SEM studies leaf surface were mounted on stub using double sided cello tape and coated with gold in a sputtering chamber (Sputter Coater BAL-TEC, SCDOOS). Epidermis do not encounter with any pretreatment. Coating with gold by the physical vapor deposition method (PVD) was restricted to 100 Å. The SEM examination was carried out on a TESCAN microscope. The measurements were based on 10-20 readings for each specimen. The terminology of Punt et al. (2007) for leaf micromorphology and Metcalfe and Chalk (1950) for stem anatomy was followed.

In order, to detect significant differences in the studied characters among studied species, Analysis of Variance (ANOVA) was performed. To reveal the species relationships, cluster analysis and principal component analysis (PCA) were used. For multivariate analysis, the mean quantitative characters were applied, while qualitative characters were coded as binary/multi-state characters. Standardized variables were used for a multivariate sta-

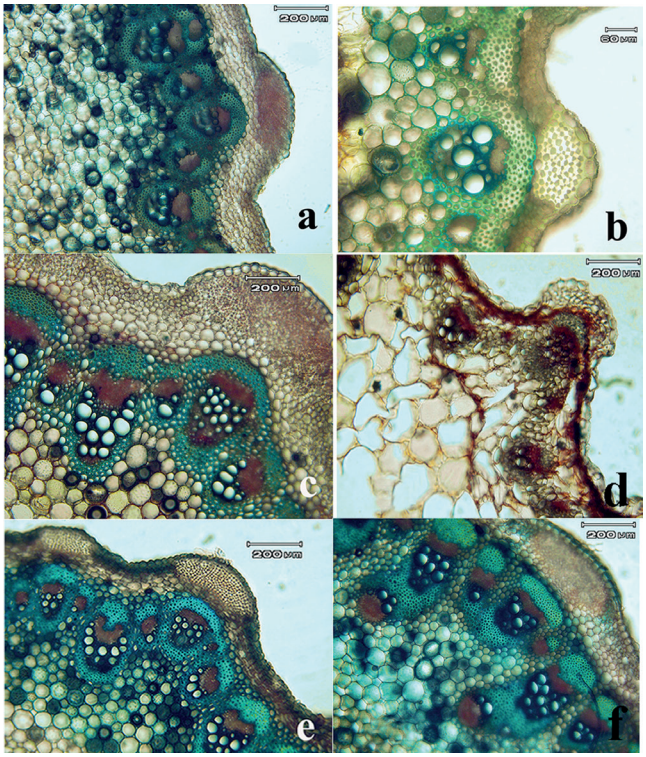

Figure 2. Stem cross section in *Rumex* species studied. a) *R. chalepensis*; b) *R. conglomeratus*; c) *R. dentatus*; d) *R. vesicarius*; e) *R. pulcher*; f) *R. elbrusensis*.

tistical analysis. The average taxonomic distances and squared Euclidean distances were applied as dissimilarity coefficient in the cluster analysis of anatomical data. In order, to determine the most variable anatomical characters among the studied species, a factor analysis based on the principal components analysis was performed. The PAST ver. 2.17c software was used for statistical analyses.

Results

Stem anatomical features

The shape of the cross-section is polygonal-protuberant, with different degree of prominence protuberances (Fig. 1). The epidermis presents cells, with the external wall thicker than the others. Epidermis is covered by a cuticle. A cord of sclerenchymatous fibers is present in the protuberances, under the stem epidermis. The central cylinder has vascular tissues arranged in one ring. Cords of sclerenchymatous fibers are visible at the periphery of each vascular bundle. The medulla is made of spongy parenchyma.

In *R. chalepensis*, ribs are short dome and regularly arranged. Parenchyma is composed of almost 8 layers and vascular bundles are in form of collateral bundles. The sclerenchymatous cap on phloem is arch-shaped (Fig. 2a). In *R. conglomerates* ribs are shaped as large domes with deep groves which are arranged regularly. There are five parenchyma layers and vascular bundles are in form of bicollateral bundles (Fig. 2b). Ribs shapes in *R. dentatus* are in different sized dome, but in *R. vesicarius*, it is in form of deeply groves small ribs (Fig. 2c). Ribs are arranged in *R. dentatus* irregularly but in *R. vesicarius* they are regular. The number of parenchyma layers is less in *R. vesicarius* than *R. dentatus*. Both showed bicollateral vascular bundles and arch-shaped sclerenchymatous cap on phloem (Fig. 2d).

Ribs in *R. pulcher* are in form of large domes which are irregularly arranged. There are 7 parenchyma layers as cortex and oxalate calcium druses crystals are present (Fig. 2e). Vascular bundles are bicollateral and the arch of sclerenchymatous cap on phloem is observed. In *R. elbrusensis* the complete sclerenchymatous sheath was not present. Only on this species medullary vascular bundle was observed. Rib shapes are in form of very short dome with regular arrangement (Fig. 2f). On the outer stem surface trichomes are observed. Cortex parenchyma is composed of 9 layers. In *R. elbrusensis* no oxalate calcium druse crystal was observed. This species has bicollateral vascular bundles. The other difference is the shape of sclerenchymatous cap of phloem, which is not arch-shaped and is in form of horizontal mass. Moreover, internal bundles can be seen in this taxon. There are also quantitative differences which are mentioned in Table 2.

Leaf epidermis micromorphology

The epidermis is made of polygonal cells with straight lateral walls; stomata, often anisocytic, are visible in both epidermis. Numerous druses of calcium oxalate are visible through the transparency. A comparison of

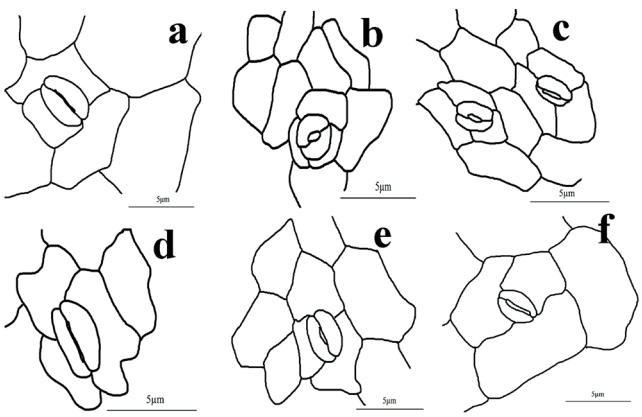

Figure 3. Leaf dorsal epidermis in *Rumex* species studied. a) *R. chalepensis*; b) *R. conglomerates*; c) *R. dentatus*; d) *R. elbrusensis*; e) *R. pulcher*; f) *R. vesicarius*. Scale bar is 5 μm.

Table 2. Results of stem cross section features in *Rumex* species studied.

Character	Taxon					
	R. chalepensis	*R. conglomeratus*	*R. dentatus*	*R. elbrusensis*	*R. pulcher*	*R. vesicarius*
Complete sclerenchyma sheath	present	absent	absent	absent	present	absent
Medullary vascular bundles	absent	absent	absent	present	absent	absent
Rib shape	short dome	large dome with deep groves	different sized dome	very short dome	large dome	deeply grooved small
Rib arrangement	regular	regular	irregular	regular	irregular	regular
Trichome on outer stem surface	absent	absent	present	present	absent	present
Parenchyma layers	8	5	7	9	7	3
Oxalate calcium druse crystals	present	present	present	absent	present	present
Vascular bundle	collateral	bicollateral	bicollateral	bicollateral	bicollateral	bicollateral
Shape of sclerenchyma cap of phloem	arch	arch	arch	horizontal mass	arch	arch
Average epidermis thickness	25.55 µm	14.60 µm	24.41 µm	18.97 µm	17.12 µm	21.14 µm
Average vascular bundles diameter	93.70 µm	105.88 µm	107.50 µm	91.01 µm	95.33 µm	79.34 µm
Average thickness of sclerenchyma fibers over phloem	43.11 µm	61.78 µm	63.24 µm	66.59 µm	50.31 µm	33.48 µm

Table 3. Comparative epidermis features in *Rumex* species studied.

Species	Stomata type		Cell size Adaxial (µm)		Cell size Abaxial (µm)		Stomata average size (µm)
	Ventral	Dorsal	Min	Max	Min	Max	
R. chalepensis	anisocytic	anisocytic	3.8	7.6	3	6.5	4.06
R. conglomeratus	anisocytic	anisocytic	2.28	3.0	2.18	6	1.995
R. dentatus	anisocytic	anisocytic	2.21	3.61	2.78	5.99	2.179
R. elbrusensis	anisoparacytic	anisocytic	4.57	4.9	3.41	5.96	3.778
R. pulcher	anisocytic	anisocytic	3.35	3.61	2.801	7.4	2.98
R. vesicarius	anisocytic	anisocytic	4.03	8.84	3.06	4.907	3.17

epidermis features in species studied in Table 3. Ventral leaf epidermis of all species showed anisocytic stomata type, although, the size of three surrounding cells and their angles are somehow varied in different species (Fig. 3). In *R. elbrusensis* stomata type is similar, to paracytic type in leaf ventral surface. The size of epidermal cells varies from 2.21 to 8.84 µm on adaxial surface and from 2.17 to 7.4 µm on abaxial surface. *R. conglomeratus* had the finest and smallest ventral epidermal cells while *R. vesicarus* has the largest one. In dorsal epidermis, *R. conglomeratus* showed the smallest cells and *R. pulcher* had the largest one. Largest stomata were observed in *R. chalepensis* while the smallest were in *R. conglomeratus* (Fig. 3).

Studying the SEM micrograph of dorsal and ventral leaf epidermis showed some details of the epicuticular wax and epidermis features. *R. chalepensis* has wrinkled cuticle on both surface epidermis. Wax is in form of smooth layers and granules. In some parts of ventral surface, a kind of striate ornamentation is observed (Fig. 4). *R. conglomeratus* showed a dorsal leaf epidermis which is composed of

Table 4. ANOVA results of quantitative anatomical and epidermal characters in *Rumex* species studied.

Source of variation	SS	Df	MS	F	P-value	F crit
Between groups	41695.88	5	8339.175	167.3471	5.75E-21	2.533555
Within groups	1494.949	30	49.83162			
Total	43190.83	35				

Table 5. PCA analysis results of anatomical and epidermal characters in *Rumex* species studied.

PCA	Eigenvalue	Percentage of variance
1	5.38628	33.664
2	4.00992	25.062
3	3.52191	22.012

Table 6. Factor analysis results of anatomical and epidermal characters in *Rumex* species studied.

Characters	Factor 1	Factor 2	Factor 3
Medullary vascular bundle	0.995	-	-
Presence/absence of oxalatecalcium crystals	0.995	-	-
Shape of sclerenchyma cap of phloem	0.995	-	-
Stomata type in ventral epidermis	0.995	-	-
Status of vascular bundle	-	0.747	-
Average vascular bundles diameter	-	0.710	-
Average thickness of sclerenchymafibers over phloem	-	0.766	-
Average size of adaxialepidermal cells	-	0.881	-
Average size of stomata	-	0.855	-
Number of parenchyma layers	-	-	0.815
Average size of abaxial epidermal cells	-	-	0.765

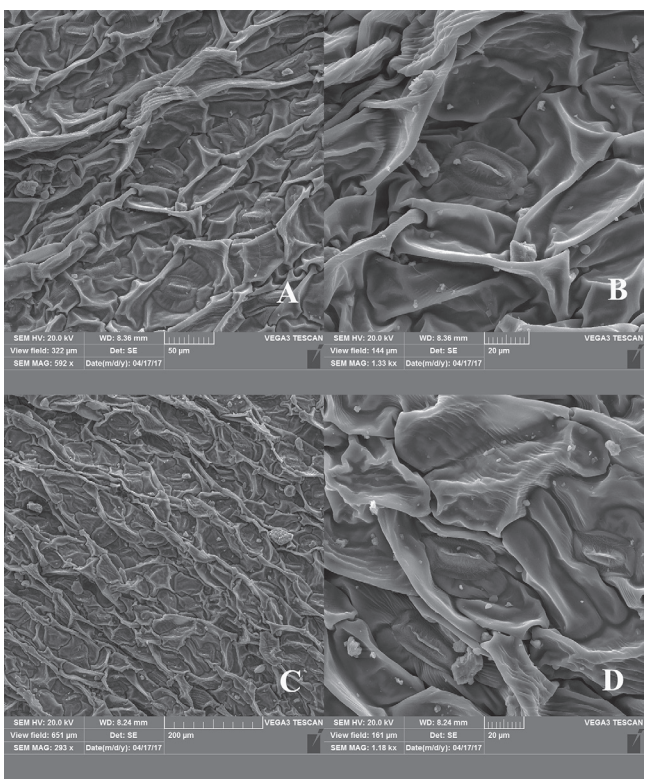

Figure 4. Leaf epidermis in *R. chalepensis*. A & B) dorsal and C & D) ventral epidermis.

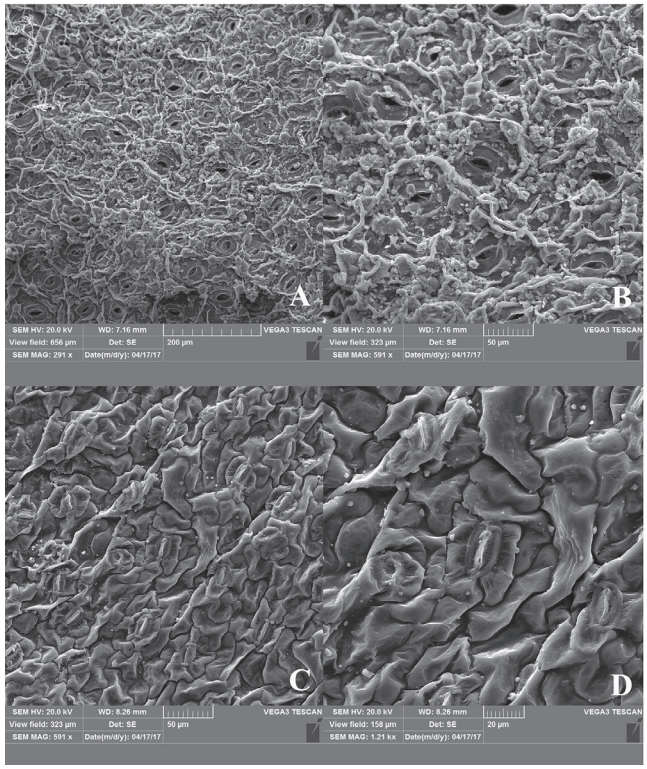

Figure 5. Leaf epidermis in *R. conglomeratus*. A & B) dorsal and C & D) ventral epidermis.

a mass of huge granules of wax and threads. A kind of stomata wax chimney was observed on dorsal epidermis too. Ventral epidermis is composed of wrinkled films of epicuticular wax. The stomata on dorsal epidermis are more frequent than ventral surface (Fig. 5).

Dorsal epidermis in *R. dentatus* demonstrated granules and smooth layer of epicuticular wax while ventral epidermis showed same situation with less granules (Fig. 6). In *R. elbrusensis* dorsal epidermis demonstrated granules and smooth layer of epicuticular wax. Ventral epidermis showed same situation with more granules (Fig. 7). *R. pulcher* showed a kind of stomata wax chimney on dorsal epidermis in addition to a mixture of granules and threads. In ventral epidermis there is no thread or granules and the stomata were less. Some kinds of platelets were observed in ventral surface (Fig. 8). In *R. vesicarius* dorsal and ventral epidermis is composed of wrinkled films of epicuticular wax with some kinds of crusts (Fig. 9).

Statistical analyses

ANOVA showed significant differences in quantitative characters (P<0.01) (Table 4). PCA analysis was done to determine the most variable characters among species studied. First three factors comprised 80.73% of total variation (Table 5). In the first PCA axis, features as

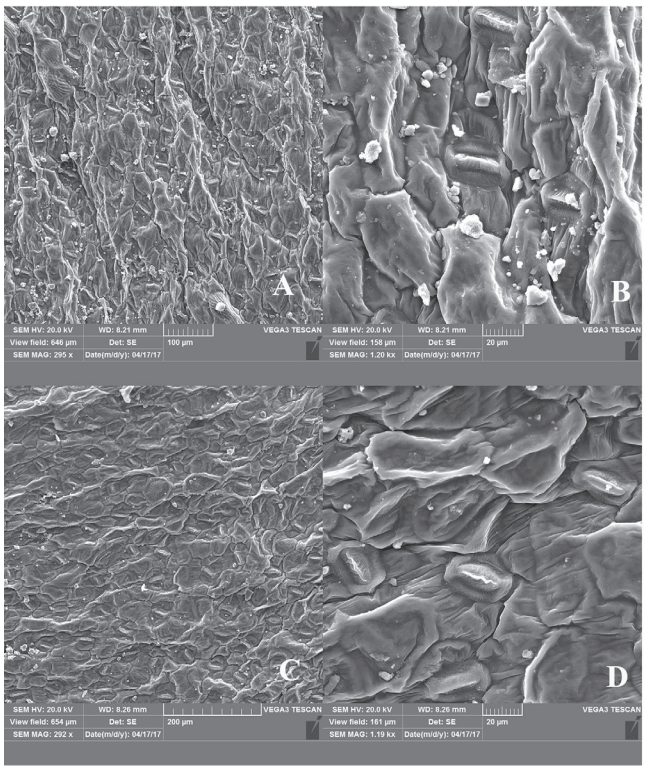

Figure 6. Leaf epidermis in *R. dentatus.* A & B) dorsal and C & D) ventral epidermis.

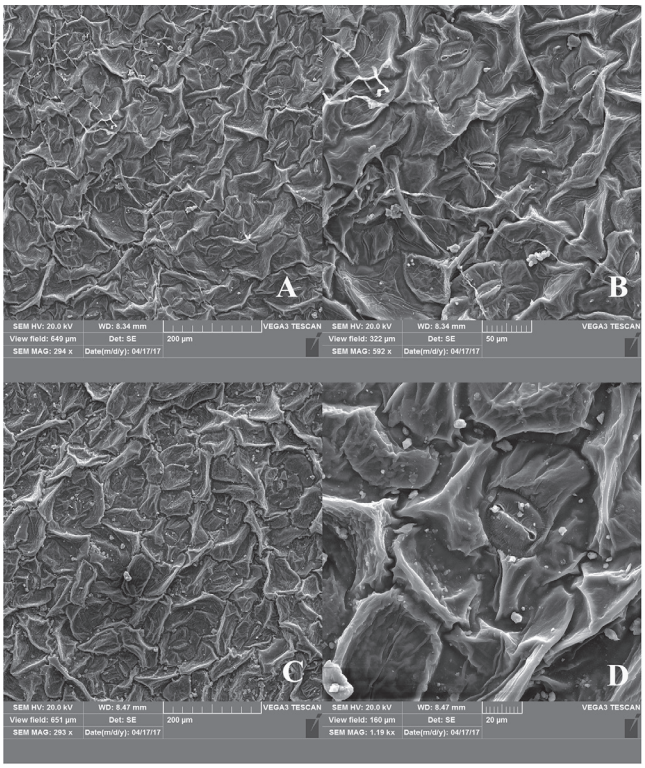

Figure 7. Leaf epidermis in *R. elbrusensis*. A & B) dorsal and C & D) ventral epidermis.

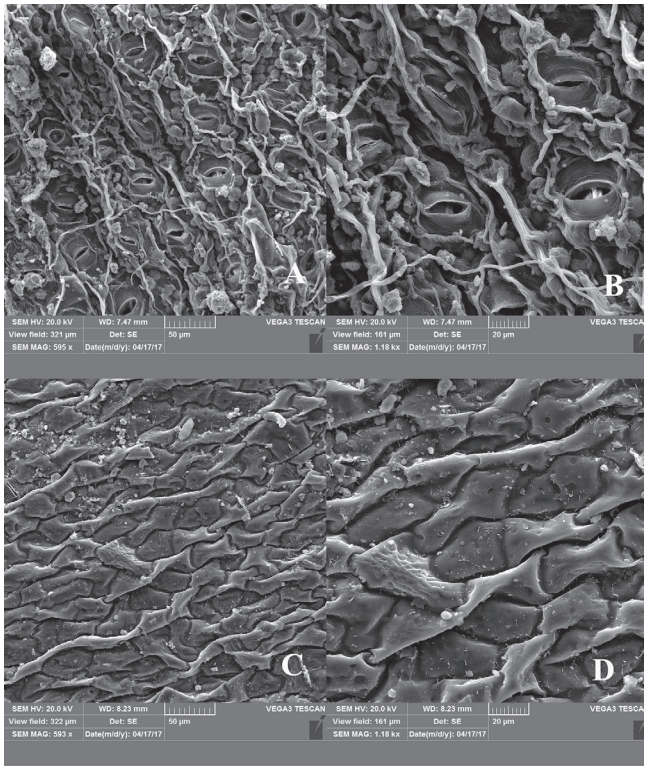

Figure 8. Leaf epidermis in *R. pulcher*. A & B) dorsal and C & D) ventral epidermis.

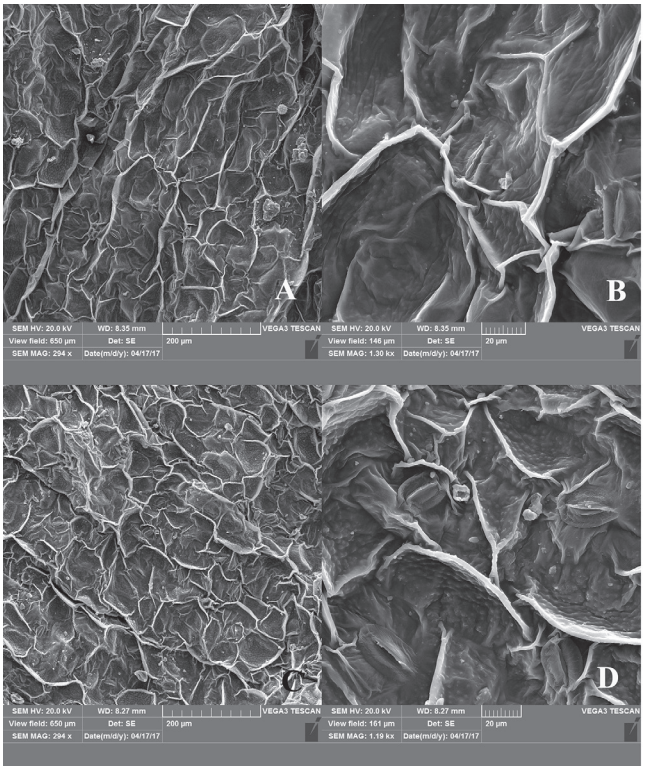

Figure 9. Leaf epidermis in *R. vesicarius*. A & B) dorsal and C & D) ventral epidermis.

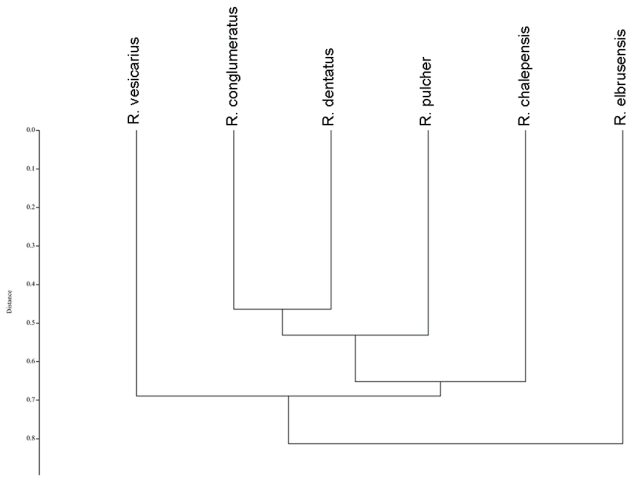

Figure 10. UPGMA tree of anatomical and micromorphological characters in *Rumex* species studies.

medullary vascular bundle, presence/absence of oxalate calcium crystals, shape of sclerenchyma cap of phloem and stomata type in ventral epidermis with 33.66% of total variation showed the highest correlation (>0.7).

Status of vascular bundle, average vascular bundles diameter, average thickness of sclerenchyma fibers over phloem, average size of adaxial epidermal cells, average size of stomata and number of parenchyma layers and average size of abaxial epidermal cells showed the highest correlation in second and third axes, respectively (Table 6).

In UPGMA tree based on all studied characters, *R. elbrusensis* was placed in a separate sub-cluster. In the second sub-cluster, *R. vesicarius* was positioned separately while other taxa grouped together (Fig. 10). Results of PCA ordination was in agreement with UPGMA tree (Fig. 11).

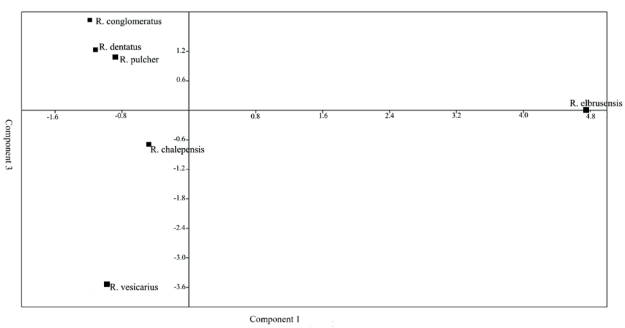

Figure 11. PCA ordination of anatomical and micromorphological characters in *Rumex* species studied.

Discussion

This investigation represents first detailed qualitative and quantitative study of leaf epidermis in *Rumex* species. It indicated the taxonomic importance of leaf epidermis in species delimitation.

Our observations showed anisocytic type of stomata in both surfaces of species studied, except *R. elbrusensis* with aniso-paracytic type in adaxial surface. Ahmad et al. (2009) reported amphianisocytic stomata pattern in *R. vesicarius*. Ayodele and Olwokudejo (2006) mentioned anomocytic and diacytic in other species of *Rumex*. Recently Yasmin et al. (2010 b) reported pericytic type for both leaf surfaces of *R. chalepensis* and *R. vesicarius* and peri-anisocytic type for *R. dentatus* which does not correspond to the present findings for the species studied. Considering epicuticular wax, species studied showed different patterns. In *R. vesicarius* wrinkled films can be seen in both sides, separating *R. vesicarius* from other taxa.

Previous studies on other Polygonaceae elements and different *Rumex* species support the usefulness of stem anatomical features in species delimitation (Hameed et al. 2010; Nazem Bokaee et al. 2015). Our studies showed the diagnostic value of these characters in studied species.

Joshi (1936) worked on the anatomy of *Rumex* with respect to the morphology of the internal bundles and the origin of the internal phloem. He suspected that absence of internal bundles in perennial *Rumex* represent the oldest forms of the genus. Among our perennial species studied (*R. elbrusensis*, *R. conglomeratus*, *R. pulcher*, *R. chalepensis*), only *R. elbrusensis* had these features therefore other perennial taxa are the oldest forms of *Rumex*. Our results were in agreement with other studies (Soleimani et al. 2014).

In both ordination and UPGMA tree, *R. vesicarius* was placed far from other species supporting its position in separate subgenus (Rechinger 1968; Mozaffarian 1988). Generally, our studies showed the taxonomic value of anatomical and micro-morphological characters in differentiation of this problematic genus.

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