Leaf anatomical studies of the annual species of *Polygonum* s.l. (*Polygonaceae*) in Iran

Maryam Keshavarzi[1,2], Samaneh Mosaferi[1] & Mohaddeseh Shojaii[1]

[1] Biology Dept., Faculty of Science, Alzahra University, Tehran, Iran
[2] Iran National Science Foundation (INSF), e-mail: neshat112000@yahoo.com (corresponding author)

Received: January 08, 2012 ▷ Accepted: June 26, 2012

Abstract. In Iran, *Polygonum* s.l. (*Polygonaceae*) comprises 13 annual species with a complex taxonomic situation. There are great morphological similarities between some of the species. Under this project, leaf anatomical studies of 38 populations from different habitats are used to distinguish different taxa. A total of 15 qualitative and quantitative morphological characters are studied. Species and relationships between populations and diagnostic value of the anatomical features are considered. Calcium oxalate crystals are found in most studied taxa. Statistical analysis confirms the diagnostic value of leaf anatomical features.

Key words: Iran, leaf anatomy, *Polygonum*

Introduction

Polygonum s.l. L. (*Polygonaceae*) is a genus with 420 species scattered across both hemispheres (Index Kewensis 1997). Rechinger & Schiman-Czeika (1968) recorded 52 species in seven sections for the Iranian Plateau, such as *Aconogonon* (Meisn.) Rchb., *Cephalophilon* (Meisn.) Spach., *Persicaria* (L.) Mill., *Bistorta* (L.) Mill., *Pleuropterus* Turcz., *Tiniaria* (Meisn.) Rchb., Webb & Moq., and *Polygonum* L. There are 32 species of *Polygonum* s.l. identified in Iran. These annual and perennial species have been of ornamental, medicinal and weed importance across the world.

Polygonum s.l. comprises annual and perennial weedy plants with a complex taxonomic situation (Ronse Decraene & Akeroyd 1988). These species have been treated in a different way in the different floras. Identification keys are mainly based on such features as homo- or heterophylly, flower color, indumentum condition, and ochrea shape and texture (Davis 1966; Rechinger & Schiman-Czeika 1968; Brandbyge 1993). Although presently some genera have been differentiated from *Polygonum*, we have considered them here in three different genera (*Polygonum, Persicaria* and *Fallopia* Adans.). Due to the high hybridization rate (Timson 1965) and phenotypic variability (Griffith & Sultan 2006), correct identification is certainly very difficult.

Ayodele & Olowokudejo (2006) have studied the members of *Polygonaceae* family by leaf anatomical features. They found some epidermal variations in the studied taxa. Epidermal cell shape and arrangement, for instance, show variation. They have provided an identification key based on epidermal features. Leaf area hairs are of taxonomic importance in this family.

Sachdeva & Malik (1986) had found that epidermal cell arrangement, outer cell walls, hair type, and midrib anatomical features are useful in the separation of *Polygonum* sections. The aim of this study was to find out the diagnostic anatomical features in the annual *Polygonum* species.

Mitchell (1971) had studied leaf morphology and anatomy of 16 species of the aquatic *Polygonum* in North America. He recognized the taxonomic importance of the midrib status, shape of collenchyma strand and types of cells in the mesophyll for delimitation of the studied taxa. Khosravi & Poormahdi (2008) have studied the

Polygonum populations of Southwest Iran. They have found out that histology and leaf anatomy could be considered significant characteristics. They have also recorded a new species on the basis of plant morphology and leaf anatomy. In this project, the leaf anatomical studies of different annual *Polygonum* s.l. species from different habitats have been used to distinguish the taxa.

Material and methods

Under this project, 38 populations of 15 annual taxa of *Polygonum* s.l. have been gathered from different habitants in Iran and studied accordingly (Table 1). From each population three specimens were taken and three replications were used for each specimen.

Table 1. Voucher details of studied annual *Polygonum* s.l species.

Species	Voucher No & collector	Locality
Polygonum argyrocoleon Steud. ex Kunze	*875 – Gholami*	Kurdistan, Sanandaj
	872 – Ghazi	Khorassan, Mashhad, Kohsangi
	864 – Gholami	Kermanshah, Kermanshah, Mahmud abad
	865 – Gholami	Kermanshah, Kasabz Jungle, Kermanshah, Gand abad
	871 – Gholami	Azerbaijan, Gougan
P. arenastrum Boreau	*861 – Rezaii nejad*	Ilam, Ilam, Chega sabz Jungle
	863 – Gholami	Tehran, Tehran, Darake.
P. aviculare L.	*892 – Nataj*	Mazandaran, Babol
	893 – Gholami	Mazandaran, Babolsar
	887 – Gholami	Azerbaijan, Boukan
	876 – Gholami	Zanjan, Takistan
	902 – Gholami	Tehran, Tehran, Saadat abad
P. olivascens Rech.f. & Schiman- Czeika	*905 – Keshavarzi*	Markazi, Saveh, Yalabad
	906 – Falatouri	Tehran, Nasim Shahr
	901 – Gholami	Tehran, Tehran, Saadat abad
	908 – Gholami	Tehran, Tehran
P. patulum M. Bieb.	*920 – Gholami*	Azerbaijan, Uromiyeh, Ayoublu
	918 – Gholami	Azerbaijan, Miandoab
	916 – Gholami	Azerbaijan, Uromiyeh, Hasanlu
P. polycnemoides Jaub. & Spach	*926 – Gholami*	Tehran, Touchal
	925 – Gholami	Tehran, Abali, Zirab
	924 – Gholami	Tehran, Darake
Persicaria hydropiper (L.) Spach	*500 – Mosaferi*	Mazandaran, Kelardasht, Gavitar village
	501 – Habibi	Mazandaran, Behshahr, Tirtash
P. maculosa Gray.	*502 – Keshavarzi*	Mazandaran, Zirab, Kechid village
P. lapathifolia ssp. *nodosa* (Pres.) Á. Löve	*504 – Mosaferi*	Hamadan, Heydareh village
P. lapathifolia ssp. *lapathifolia1* L.	*506 – Gholami*	Kermanshah, Kermanshah, Gharesoo River margin
	511 – Mosaferi	Tehran, Tehran, Vanak
P. lapathifolia ssp. *brittingeri* (Opiz) Soják	*513 – Amini*	Mazandaran, Noushahr
P. minor (Huds.) Opiz	*514 – Mosaferi*	Isfahan, Golpaygan, Saravar village
	515 – Mosaferi	Alborz, Karaj, Jahanshahr
	522 – Mosaferi	Mazandaran, Kelardasht, Gavitar village
	523 – Mosaferi	Alborz, Karaj, Chamran Park.
	519 – Keshavarzi	Guilan, Anzali to Lahidjan, Chaparpord village
P. mitis (Schrank.) Holub	*534 – Nataj*	Tehran, km 4 to Rasht
	535 – Mosaferi	Mazandaran, Abbas Abad, Abbas Abad forest
Fallopia convolvulus (L.) Á. Löve	*221 – Keshavarzi*	Tehran, Evin
F. dumetorum (L.) Holub.	*225 – Mosaferi*	Tehran, Tehran

The species were determined according to various floras (Hooker 1885; Webb & Chater 1964; Rechinger & Schiman-Czeika 1968). Vouchers were deposited in the Herbarium of Alzahra University (AUH). Samples were taken from the middle part of the leaf blade. Leaf materials were prepared by manual cutting. Cross sections were immersed in 10 % of hydrogen peroxide for 20 minutes for bleaching, then washed and stained with methyl green and Congo red. Photos were taken by a light microscope with an Olympus DP12 digital camera. The quantitative characteristics were measured by UTHSCSA Image tool, version 3.0 (2002). The terminology of Cullen (1978) and Stearn (1983) was followed for the general outline of leaf cross section.

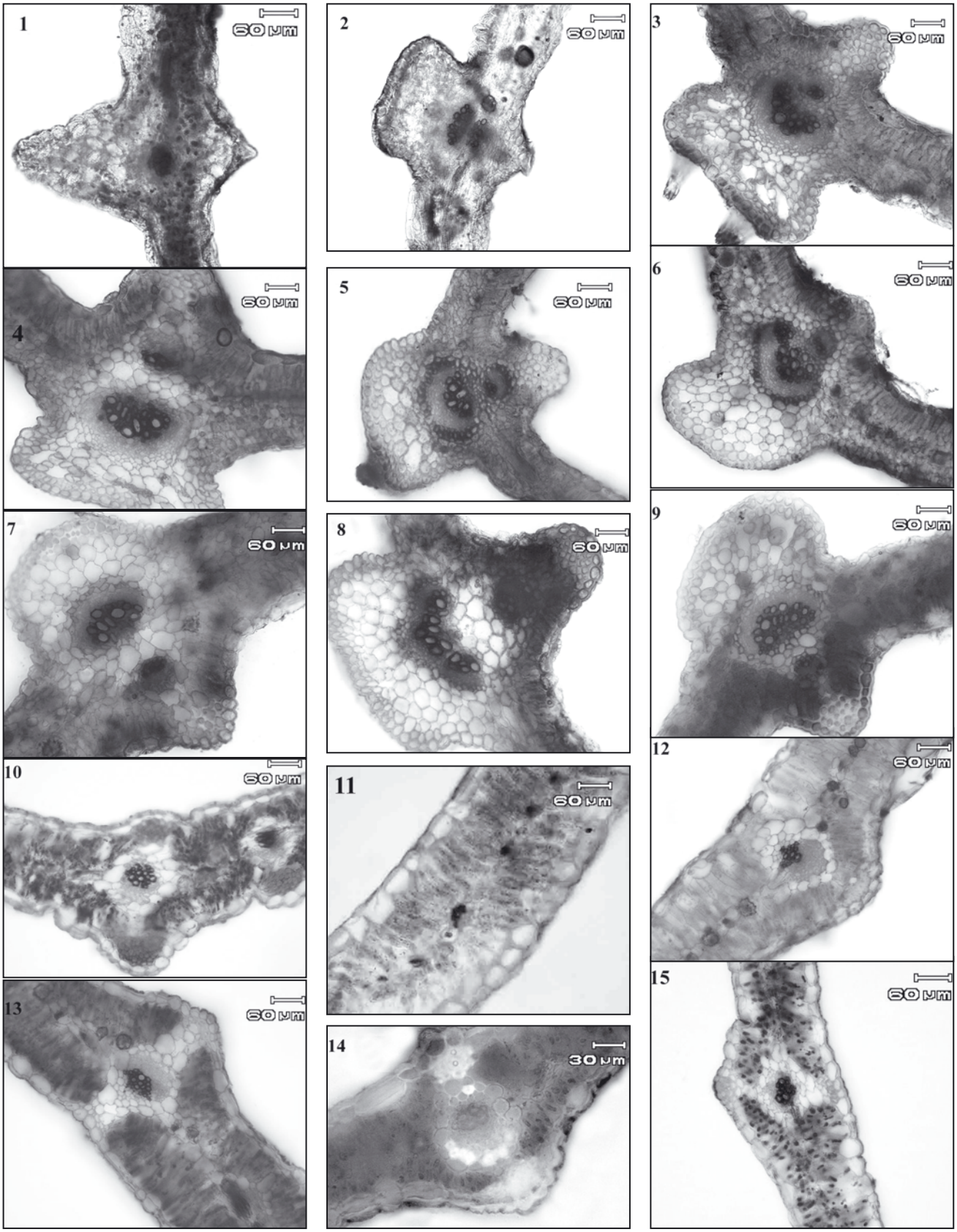
variance analysis (ANOVA) was applied to detect the significant differences in the studied characters of various species. To reveal the species relationships, we used cluster analysis and principal component analysis (PCA) (Ingrouille 1986). For multivariate analysis, the mean values of the quantitative characters were used, while the qualitative characters were coded as binary/multi-state characters. Standardized variables were used for a multivariate statistical analysis. The average taxonomic distances and squared Euclidean distances were used 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. We have used SPSS, ver. 9 (1998) software for statistical analysis.

Results

The anatomical leaf structure studied in the *Fallopia* species has revealed that in *F. convolvulus* leaf margins were wider than in *F. dumetorum* (Figs 1, 2). Collenchyma strands had the same length but different shape in the *Fallopia* species. In *F. dumetorum* it was oblong, while in *F. convolvulus* it was embowed. In both species mesophyll was composed of palisade cells. No vascular bundle sheath and fibers have been observed in these species (Table 2).

Table 2. Qualitative and quantitative leaf cross section characteristics of the studied *Polygonum* s.l. species

Taxon	Width of leaf blade	Length of vessel	Width of vessel	Mean width of mesophyll cells	Length of collenchyma strand	Length of ventral expulsion	Collenchyma shape	Mesophyll cell	Parenchyma cell	Calcium oxalate crystal	Vascular bundle fibers	Vascular bundle sheet	Ventral situation of midrib	Dorsal situation of midrib
Polygonum argyrocoleon	128.90	53.82	58.35	17.75	63	16.61	embowed	palisade	rounded	present	present	indistinct	swollen	splint
P. arenastrum	227.89	69.30	42.55	26.53	52.39	0	embowed	palisade	rounded	present	present	distinct	swollen	flat
P. aviculare	268.42	85.16	88.59	29.50	49.65	0	oblong	palisade	rounded	present	present	distinct	swollen	flat
P. olivascens	160.90	54.53	60.80	21.66	35.34	15.60	embowed	palisade	angled	present	absent	indistinct	splint	splint
P. patulum	193.79	66.16	49.70	24.14	40	15.55	oblong	palisade	angled	present	absent	indistinct	splint	splint
P.polycnemoides	265.47	46.56	44.88	28.12	0	0	short	palisade	rounded	absent	absent	indistinct	flat	flat
Persicaria hydropiper	168.01	109.94	155.36	22.89	60.81	159.32	discoid	spongy	rounded	present	present	indistinct	swollen	swollen
P. maculosa	243.52	116.34	140.83	32.33	129.95	287.89	embowed	palisade	angled	absent	present	indistinct	swollen	swollen
P. lapathifolia ssp. *nodosa*	129.09	91.07	148.50	23.66	96.87	166.22	oblong	both	angled	present	present	indistinct	swollen	swollen
P. lapathifolia ssp.*lapathifolia*	168.72	85.30	170.62	19.07	136.38	133.77	oblong	both	angled	present	absent	indistinct	swollen	swollen
P. lapathifolia ssp. *brittingeri*	172.59	107.29	175.52	19.79	137.03	89.99	oblong	both	angled	present	absent	indistinct	swollen	swollen
P. minor	184.30	110.85	161.65	28.61	94.15	152.25	oblong	spongy	rounded	absent	present	indistinct	swollen	swollen
P. mitis	147.12	89.14	221.66	30.88	105.72	107.72	strongly oblong	palisade	rounded	present	present	indistinct	swollen	swollen
Fallopia convolvulus	151.82	52.73	32.73	25.09	54.54	159.39	embowed	palisade	rounded	absent	absent	indistinct	swollen	swollen
F. dumetorum	128.80	71.61	31.82	17.33	54.55	102.73	oblong	palisade	rounded	present	absent	indistinct	swollen	swollen



Figs 1-15. Leaf cross section in *Polygonum* s.l. **1**, *Fallopia convolvulus*; **2**, *F. dumetorum*; **3**, *Persicaria lapathifolia* subsp. *lapathifolia*; **4**, *P. lapathifolia* subsp. *brittingeri*; **5**, *P. lapathifolia* subsp. *nodosa*; **6**, *P. hydropiper*; **7**, *P. maculosa*; **8**, *P. mitis*; **9**, *P. minor*; **10**, *Polygonum patulum*; **11**, *P. polycnemoides*; **12**, *P. arenastrum*; **13**, *P. aviculare*; **14**, *P. argyrocoleon*; **15**, *P. olivascens*.

In the studied *Persicaria* species, *P. maculos*a had wider leaf margins in relation to other *Persicaria* species. The collenchyma strand in *P. mitis* was strongly oblong and has not been observed in any other studied species. Furthermore, the midrib in the dorsal part of this taxon was square-shaped, contrary to other *Persicaria* species with trapezoid (*P. lapathifolia* ssp. *brittingeri*) or round-shaped midribs. In *Persicaria,* the leaf anatomical structures contained intercellular lacunae in the mesophyll tissue (except *P. maculosa* and *P. mitis*) found only in the *Persicaria* species. No vascular bundle sheet has been observed in these species (Figs 3-9).

The studied *Polygonum* species have shown some differences in their leaf anatomical structure. In *P. argyrocoleon* the leaf margin was widest (128.90 μm). Contrary to other *Polygonum* species, in *P. polycnemoides* the collenchyma strands were very tiny and indistinct. No oxalate calcium crystals have been observed. Although in most studied *Polygonum* species there have been only dorsal collenchyma strands, in some *P. patulum* accessions there were adaxial and abaxial collenchyma strands (Figs 10-15).

The studied species have differed significantly in most quantitative characters, as revealed by the ANOVA test. Cluster analysis and PCA ordination of the *Polygonum* s.l. species of Iran, based on both quantitative and qualitative anatomical characters, have produced similar results (Figs 16, 17). In cluster analysis, two major clusters were formed. The first major cluster comprised two subclusters: with populations of *P. lapathifolia* subspecies in the first one, and with the other *Persicaria* species, except for *P. maculosa*, nesting near *P. lapathifolia* subspecies in the second one.

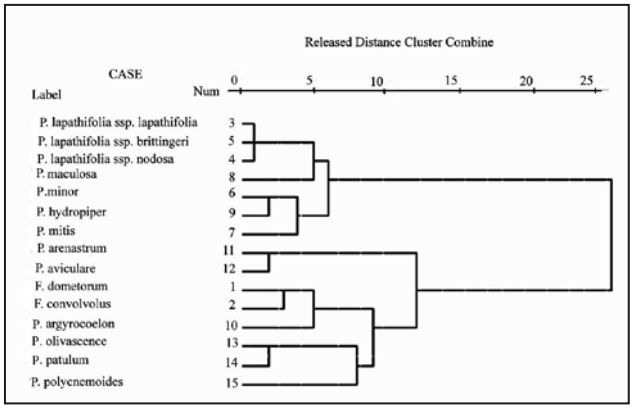


Fig. 16. Phenogram obtained by WARD method on the basis of leaf anatomical characters in *Polygonum* s.l.

Evidently, the different *P. lapathifolia* subspecies have been closely related. The studied *Fallopia* species have been closely related too. They both were related to *P. argyrocoleon*. *P. aviculare* and *P. arenastrum* and were considered closely related to each other on the basis of the leaf anatomical features. The clusters made it evident that *Persicaria*, *Fallopia* and *Polygonum* have been clearly separated anatomically.

In order to determine the most variable characters of the studied species, a Factor Analysis based on PCA has been performed, revealing that the first four factors comprised over 77.25 % of the total variation. In the first factor with about 33.70 % of total variation (Table 3), such characters as the width of vessels, length of collenchyma strands, length of vessels, length of ventral expulsion, and dorsal situation of midrib have shown the highest correlation (>0.7).

In the second factor with over 18.73 % of total variation, the width of mesophyll cells has shown the highest correlation. The third factor with 13 % of total variation and comprising the calcium oxalate crystals has featured the highest correlation. Therefore, these

Table 3. Factor Analysis results based on the anatomical characters of *Polygonum* s.l. populations in Iran

Character	Factor 1	Factor 2	Factor 3
Width of vessels	0.863	–	–
Length of collenchyma strands	0.861	–	–
Length of vessel	0.886	–	–
Length of ventral expulsion	0.795	–	–
Dorsal situation of midrib	0.830	–	–
Width of mesophyll cells	–	0.685	–
Calcium oxalate crystal	–	–	0.756

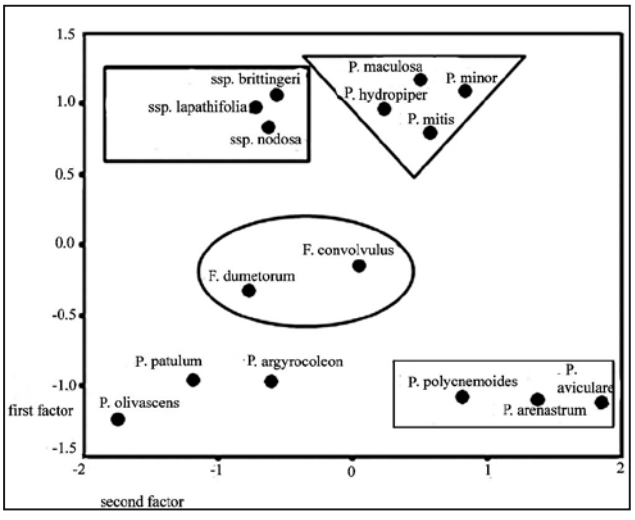


Fig. 17. PCA ordination of leaf anatomical characters in *Polygonum* s.l.

are the most variable anatomical characters among the studied *Polygonum* s.l. species of Iran.

Multivariate analysis methods have proved very helpful in assessing the inter-specific affinities and intra-specific variability of a complex group of species. Such methods were used by Ng & al. (1981) in the studies of rice species aimed at clarifying the inter-specific relationships and at distinguishing the species or geographical forms. The use of similar methods in the present study has indicated that three subspecies of *P. lapathifolia* form sister groups (Fig. 16).

Discussion

Our observations have indicated vast distribution of oxalate calcium crystals in most of the studied *Polygonum* s.l. species. Metcalfe & Chalk (1979) and Lersten & Curtis (1992) believed that this is a common feature of the *Polygonum* species.

Mitchell (1971) revealed that the collenchyma shape in leaves was of taxonomic importance, especially when the studied cross sections were made from basal leaf parts. Our results are in concordance with his findings. He also pointed out the angular shape of collenchymas in most immature leaves of the *Polygonum* species, while there were lamellar collenchyma strands in the mature ones. We have observed mainly lamellar collenchymas.

Mitchell also had found palisade mesophyll penetration in the collenchyma tissue. such penetration was not observed in our studies. He explained intercellular lacunae and their presence by the aquatic environment. In the present study, such lacunae have only been observed in the *Persicaria* species. Considering the fact that *Persicaria* are almost semiaquatic elements and that we have gathered them besides the river banks, such explanation seems incredible.

Fahn (1952) recognized oblong palisade mesophyll cells in *Polygonum* nearly squeezed without intercellular lacunae. Khosravi & Poormahdi (2008) have also mentioned such characteristics. Our results are in concordance with these findings. Furthermore, Fahn (1952) mentioned the presence of bundle fibers in the studied species. Our results also confirm his findings.

In cluster analysis (Fig. 16), the *Persicaria* species have been placed in a separate cluster, corresponding to an earlier study (Mosaferi & Keshavarzi 2011). Furthermore, similarity of the *P. lapathifolia* subspecies found out under this project has confirmed some earlier studies (Mosaferi 2010; Mosaferi & al. 2011).

The close relation of *P. patulum* and *P. olivascens* and the status of *P. arenastrum* and *P. aviculare* taxa were predicable, owing to earlier findings (Gholami 2009; Shojaii 2011).

Acknowledgments. The authors wish to thank the Iran National Science Foundation (INSF) for the financial support of this research.

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