Comparative anatomy of the *Fumaria* (*Papaveraceae*) species in Iran

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Abstract. *Fumaria* is an annual genus distributed in different habitats of Iran. The species have been usually described on the basis of sepal size, corolla and fruit characters. The aim of this study is to investigate whether the anatomy of stem, root and fruit may help clarify the relationships and delimitation of the Iranian *Fumaria* representatives. This is the first anatomic study of *Fumaria* in Iran. The results have shown some differences in the anatomical features between the species. Among these, the shape of stem cross sections and the number of vascular bundles have shown some variations, as well as the rib thickness, wing shape and wall texture of fruits. The anatomical root structure was homogenous. The results contributed to the conclusion that the anatomical features of fruits are of diagnostic value for the *Fumaria* species of Iran. As *F. parviflora* and *F. vaillantii* are recorded as tetraploid (2n=32), the high range of anatomical variation in these taxa seems to be a consequence of their ploidy level. Anatomical observation of stem and root cross-sections has revealed that symmetry, presence or absence of angles in the cross section, and xylem shape are of diagnostic importance. Results of the anatomical study of fruits reveal that some anatomical features can be used to differentiate the species with high morphological similarities, such as *F. vaillantii*, *F. parviflora* and *F. indica*.

Key words: fruit anatomy, *Fumaria*, Iran, root anatomy, stem anatomy

Introduction

Subdivision of modern *Papaveraceae* seems to be still controversial. Lidén (1986) followed Thorne (1977), who recognized seven subfamilies. Miller (1754) established the genera *Capnoides*, *Cysticapnos* and *Capnorchis* (=*Dicentra*), but kept *Corydalis* within *Fumaria*. Medikus (1789) added *Pseudofumaria* and substituted *Cysticapnos* with *Corydalis*. Both these names, together with *Capnoides*, were subsequently used to designate all taxa with many-seeded fruit, in contrast to *Fumaria*.

Pugsley (1919) classified the *Fumaria* species in two sections on the basis of flower size (*Grandiflora* and *Parviflora*), with three subsections each. Our studied species fall into sect. *Parviflora*. *F. schleicheri*, *F. indica, F. vaillantii,* and *F. parviflora* are in the *microsepalae* subsection; *F. densiflora* is in subsection *Latiosepalae,* and *F. officinalis* in subsect. *Officinalis*. Soler (1983) also referred the *Fumaria* species to two sections: sect. *Grandiflorae* and sect. *Fumaria*. The species studied in the present article belong to the *Fumaria* section, according to his classification. *F. officinalis* and *F. densiflora* fall into the *Fumaria* subsection, while, *F. parviflora* and *F. vaillantii* fall into *Microsepalae*.

In his comprehensive monograph Lidén (1986) referred the *Fumaria* species to *Fumarioideae, Fumarieae, Fumariinae*. This genus was given with two sections: *Caperolata* and *Fumaria*. The present-

ly studied species are classified into two groups: *Officinalis* (*F. officinalis* and *F. densiflora*) and *Parviflora* (*F. schleicheri, F. indica*, *F. vaillantii, F. parviflora* and *F. asepala*).

So far it is evident that *F. officinalis* and *F. densiflora* have been always referred to separate classification positions contrary to the *Microsepalae* species. Wilson & al. (1990) have subjected some *Fumaria* species to a phenetic study. They also revised some of the earlier classifications. They resorted to ordination study for section delimitation. Certain incongruence was found between the phenetic results and the classification proposed by Pugsley (1919). A close relationship between *F. parviflora* and *F. vaillantii* was indicated. The results were partially unrelated to the Iranian *Fumaria* species. Lidén (1991) expressed disagreement with the opinion of Wilson & al. (1990). He believed that, after resorting to a phenetic approach, a taxonomist in principle cannot be wrong in the absence of an underlying model. The relationships in *Fumaria* are strongly reticulate and largely impossible to be illustrated in a dichotomous dendrogram (Lidén 1991).

The genus *Fumaria* L. includes eight species distributed in different habitats of Iran (Wendelbo 1974; Lidén 2000). The strong morphological similarity of the Iranian *Fumaria* species may reflect their close phylogenetic relationship. This hypothesis however is not easy to verify, because strong modification after the collection of specimen of *Fumaria* species occasionally makes their identification very difficult, or even impossible. This is especially true for such species pairs like *F. asepala* Boiss. – *F. indica* (Hauskn) Pugsley and *F. vaillantii* Loisel. – *F. parviflora* Lam.

Fruit anatomy has proved itself useful in providing valuable characters for establishing taxonomical boundaries in many plant groups (e.g. Liu & al. 2006; Souza & al. 2008). Amongst these characters stand out the thickness of carpel layers, crystal, mericarp surface and ornamentations in transversal section (TS), as well as vascular bundle position (Budantsev & Lobova 1997; Sukhorukov 2007). Within the subfamily *Fumarioideae* in the Papaveracae family (APG III 2009), Japanese representatives of the genera *Corydalis* and *Dicentra* have been extensively investigated from the viewpoint of their seed coat anatomy (Fukuhara 1992). The nine seed coat types described in that study were used as a key for establishing the infrageneric classification of those taxa.

Kiliç & al. (2006) has studied the anatomical structure of root, stem and leaf of some *Corydalis* species. Their results have shown that the number and size of vascular bundles in the stem cross sections are taxonomically important, but no diagnostic characters were found in root and leaf cross sections. Anatomical structure of fruits has provided a wider range of carpel characters used for differentiation of species, even if the fruits were immature (Brückner 1992).

The interest in the *Papaveraceae* family, in which *Fumaria* is included, has considerably increased with the use of such tools as evolutionary-developmental biology, which are opening a new avenue for understanding the morphological characters (Hidalgo & Gleissberg 2010, and references therein). Data for species with zygomorphic flowers, such as *Fumaria*, are especially needed (Hidalgo & Gleissberg 2010). These include detailed morphological and anatomical surveys, which are very deficient for *Fumaria*. In fact, there is very scanty data from the anatomical studies of *Fumaria* species in Iran or other parts of the world. In this project the anatomical structure of stem, root and fruit in the different species of *Fumaria* in Iran is studied for the first time.

Material and methods

Twenty different populations of seven *Fumaria* species were studied from the viewpoint of their stem and root anatomy (Table 1). All studied vouchers are deposited in Alzahra Herbarium, Tehran, Iran (Table 1 and Fig. 1). For each specimen, proper replications were used. Anatomical structures of stem were studied by their quantitative features, such as the number of vascular bundles, mean stem, pith and cortex diameter in cross section, ratio of xylem to phloem bundles, stele to stem diameter, pith to stem diameter, and cortex to stem diameter (Table 2).

Root cross section was studied by such quantitative features as the ratio of xylem to phloem bundle, root diameter, stele to root diameter, and cortex to root diameter (Table 2).

Fruit specimens were gathered from nature during the period 2009–2010. 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.

Fig. 1. Locations of collection of some *Fumaria* species in this study. Legend: **X** – *F. schleicheri*, ⬬ – *F. asepala*, ★ – *F. indica*, ✻ – *F. officinalis*, ■ – *F. parviflora*, ● – *F. vaillantii,* and **O** – *F. densiflora*.

Table 1. List of studied taxa and vouchers.

Taxon	Locality	Collector	Herb. No
F. asepala Boiss.	Chahar mahale bakhtiyari, Gandoman	Yazdanbakhsh	8654
	Tehran, Firouzkoh, Amin abad	Keshavarzi	8655
	Tehran, Taleghan	Falatouri	8657
F. densiflora DC.	Mazandaran, Kelardasht	Ebrahimzadeh	8659
F. indica (Hausskn.) Pugsley	Tehran, Chitgar	Ebrahimzadeh	8635
F. officinalis L.	Mazandaran, Gelogah	Nataj	8661
F. parviflora Lam.	Lorestan, Dashte Chegeni	Direkvandi	8642
	Golestan, Golestan national Park	Ebrahimzadeh	86n1
	Yazd, Taft	Keshavarzi	8648
	Tehran, Vanak	Ebrahimzadeh	8636
	Tehran, Abali, Mobarak abad	Nataj	8639
	Yazd, Yazd	Keshavarzi	8647
F. schleicheri Soy.-Will.	Kerman, Sirjan	Habibi Tirtash	8901
F. vaillantii Loisel.	Hamedan, Heydare	Keshavarzi	8628
	Firouzkoh, Gadok	Keshavarzi	8612
	Tehran, Vanak, Modiriat bridge	Ebrahimzadeh	8626
	Mazandaran, Abeask	Ebrahimzadeh	8610
	Mazandaran, Veresk	Ebrahimzadeh	8611
	Tehran, Jajroud	Ebrahimzadeh	8614
	Tehran, Vanak	Ebrahimzadeh	8615
	Tehran, Gachsar	Rastipisheh	8627

Table 2. Studied stem and root anatomical features.

Quantitative features	
1 Stem vascular bundles number	2 Stem pith diameter
3 Stem cortex diameter	4 Stem stele diameter
5 Stem diameter	6 Root diameter
7 The ratio of xylem to phloem in root	
Qualitative features and their state of character	
1 Stem cross section shape	Distinctly angled, not angled
2 Stem cross section	Symmetrical, asymmetrical
3 Stem cross section	Rounded, elliptical
4 Root xylem shape	Fan shaped, not so

In order to detect significant differences in the studied characters among the various studied species, a variance analysis (ANOVA) was performed (Appendix 1). To reveal the species relationships, a cluster analysis and a principal component analysis (PCA) were used (Ingrouille 1986). 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 statistical 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. We used SPSS ver. 9 (1998) software for statistical analysis.

Results

The quantitative and qualitative anatomical characteristics of stem, roots and fruits of the Iranian *Fumaria* species show a certain level of variation. Stem cross sections revealed that stem angles in TS and cross section symmetry are variable within the group of studied species.

In terms of roots, cross sections revealed that the xylem bundles shape varies in different studied *Fumaria* species.

General anatomical features in the genus *Fumaria*

Stem cross sections showed a symmetric or subsymmetric pentagonal general shape, with distinct angles or not. Owing to the age of individuals, the collenchymas density varied in the angles. The thickness of cortex layer under the epidermis differed between the species

Table 3. Comparative results of stem and root anatomical features in studied *Fumaria* species.

Taxa	TS shape Stem	Cortex Diam.	Vb No	Pith Diam	Hollow Stem	TS root shape	Vb shapes
F. asepala	Round with a beak	278.6	10	475	±	Round to elliptic	Ring
F. densiflora	Pent-angular and asymmetrical	345	10	655	+	Elliptic	Fan shape
F. indica	Round	153	9	508	+	Rounded	Fan shaped
F. officinalis	Pent-angular and symmetrical	152	10	773.5	+	Elliptic	Fan shaped
F. parviflora	Pent-angular with symmetry or without	172	11	580	±	Round to elliptic	Ring
F. schleicheri	Pent-angular and asymmetrical	136	10	777.2	–	Round	Ring
F. vaillantii	Pent-angular with symmetry or without	177	9	748	±	Round to elliptic	Ring

Abbreviations: **TS:** Transversal section, **Vb:** Vascular bundle.

(Fig. 2). The number of vascular bundles and pith diameters also varied among the studied taxa (Table 3).

Root cross sections showed that anatomical structure in all studied taxa was homogenous. The shape of cross sections was circular to elliptic. One or two parenchyma layers encircled them. The difference in cortex thickness may be due to the plant age at the moment of specimen collection. In stele, the ratio between xylem and phloem bundles varied in the different *Fumaria* species (Fig. 3). Vascular bundles constituted a complete ring or not (Table 3).

Observations of fruit anatomy

In this research we have studied the anatomical structure of fruit in seven species of *Fumaria*. The similarities between the populations of species have been evaluated. In this genus fruit is an achene. Fruit shape was different in each species. It was cordate in *F. officinalis* and this feature was of diagnostic value. In other species, the fruits were circular and elliptic in cross section and were not of diagnostic importance. Other important features of fruit with diagnostic values were the apical shape of fruit, amount of fruit notch, and presence or absence of keel.

In all TS of the fruit wall we have observed 4–5 layers. The endocarp was composed of one to many layers of sinuate cells and was very thin, but getting thickened under the sclerenchymatous layer at the fruit tip, and kind of folded on the inner side of the fruit. There were some differences in that layer between the investigated *Fumaria* species. The mesocarp was strongly sclerenchymatous and its thickening varied according to the species. The sclerenchymatous layer of the mesocarp had echinate ornamentations. The different species had long, short, double, or apically curved projectons. In some species dimorphic or monomorphic projections have been observed. Apparently, the spine shapes were of taxonomic value (Fig. 4). The outer layer of the fruit was composed of large epidermal cells, with cutinized cell walls and some hypodermal cells. The thickness of that layer did not differ in the *Fumaria* species of Iran (Fig. 5 and Table 4).

A cluster analysis has revealed that *F. vaillantii* and *F. parviflora* are closely related (Fig. 6). The cluster analysis and PCA ordination of the *Fumaria* species of Iran, based on both quantitative and qualitative anatomical characters, have produced similar results (Fig. 7). Two major clusters were formed. The first major cluster comprised two subclusters, in the first of which fell *F. vaillantii* and *F. parviflora*. The other species in this group were *F. officinalis* and *F. schleicheri*. They were both related to *F. parviflora and F. vaillantii*. In the other main cluster *F. asepala* and *F. indica* were more similar to each other, and *F. densiflora* formed a separate cluster.

In order to determine the most variable anatomical characters among the studied *Fumaria* species, a factor analysis based on PCA was performed. It revealed that the first four factors comprised over 86 % of the total variation. In the first factor, with about 35 % of the total variation, such characters as stem pith diameter, stem symmetry, average stem diameter, root diameter and stem cross section shape had the highest correlation (≥0.7). In the second factor, with about 24 % of the total variation, characters as stem cortex and pith and stele diameter showed the highest correlations. Vascular bundle shape was the most important character in the third factor. Therefore, these were the most variable anatomical characters among the studied *Fumaria* species of Iran.

As it is evident from the PCA graph, each of the seven studied species has been distinctly separated (Fig. 7). Inspite of morphological similarities, the studied anatomical characters revealed that *F. schleicheri* was distinctly separated from *F. vaillanti* and *F. parviflora* and these two from the other. *F. densiflora* and *F. officinalis* showed a separate position in the graph which is in concordance with morphological observations.

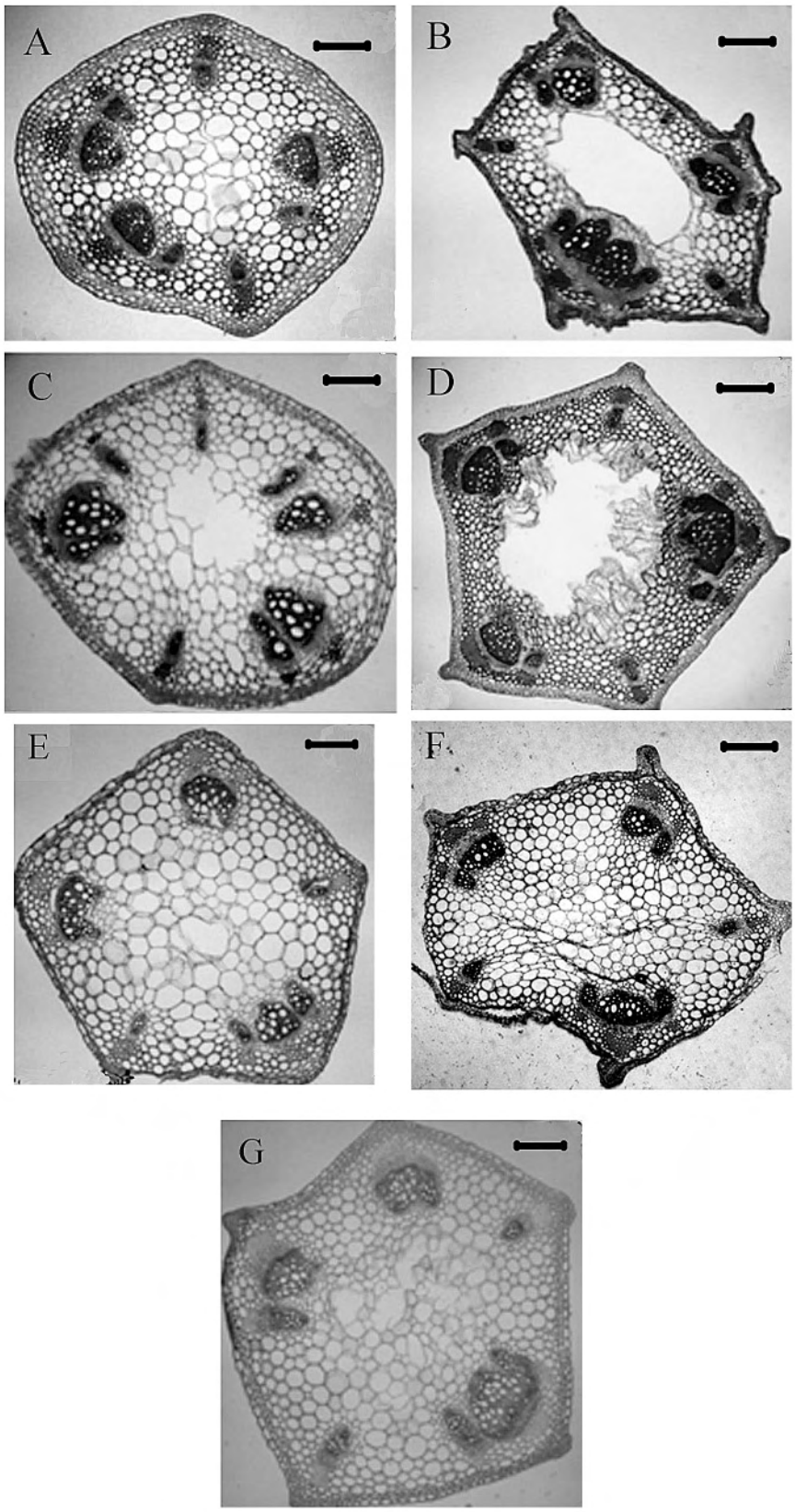

Fig. 2. Stem cross sections in the different studied *Fumaria* species: **A,** *F. asepala*; **B,** *F. densiflora*; **C,** *F. indica*; **D,** *F. officinalis*; **E,** *F. parviflora*; **F,** *F. scelcheri*; **G,** *F. vaillantii.* Scale bar, 300 μm.

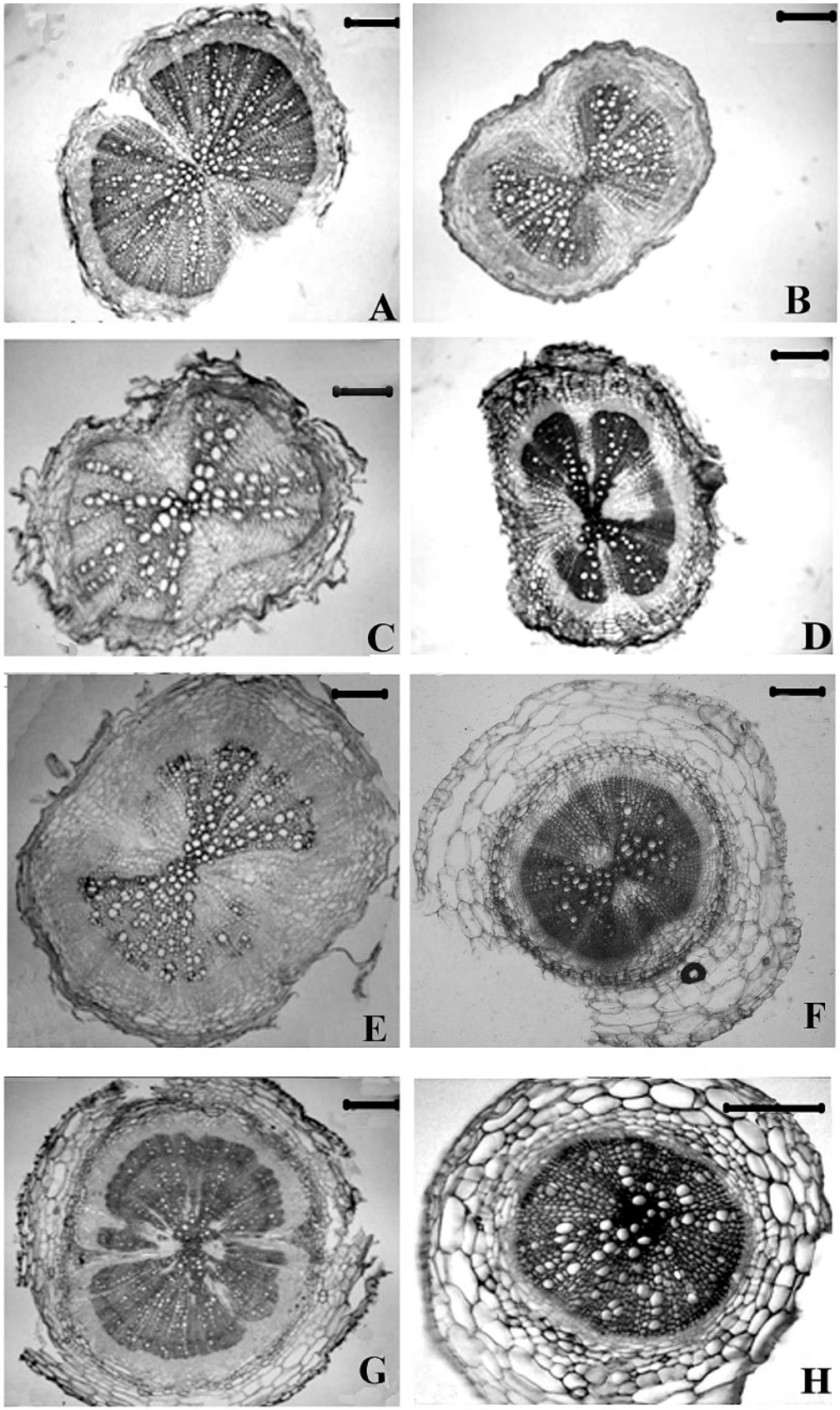

Fig. 3. Root cross sections in the different studied *Fumaria* species: **A,** *F. asepala*; **B,** *F. densiflora*; **C,** *F. indica*; **D,** *F. officinalis*; **E,** *F. parviflora*; **F,** *F. scelcheri*; **G & H,** *F. vaillantii*. Scale bar, 300 µm.

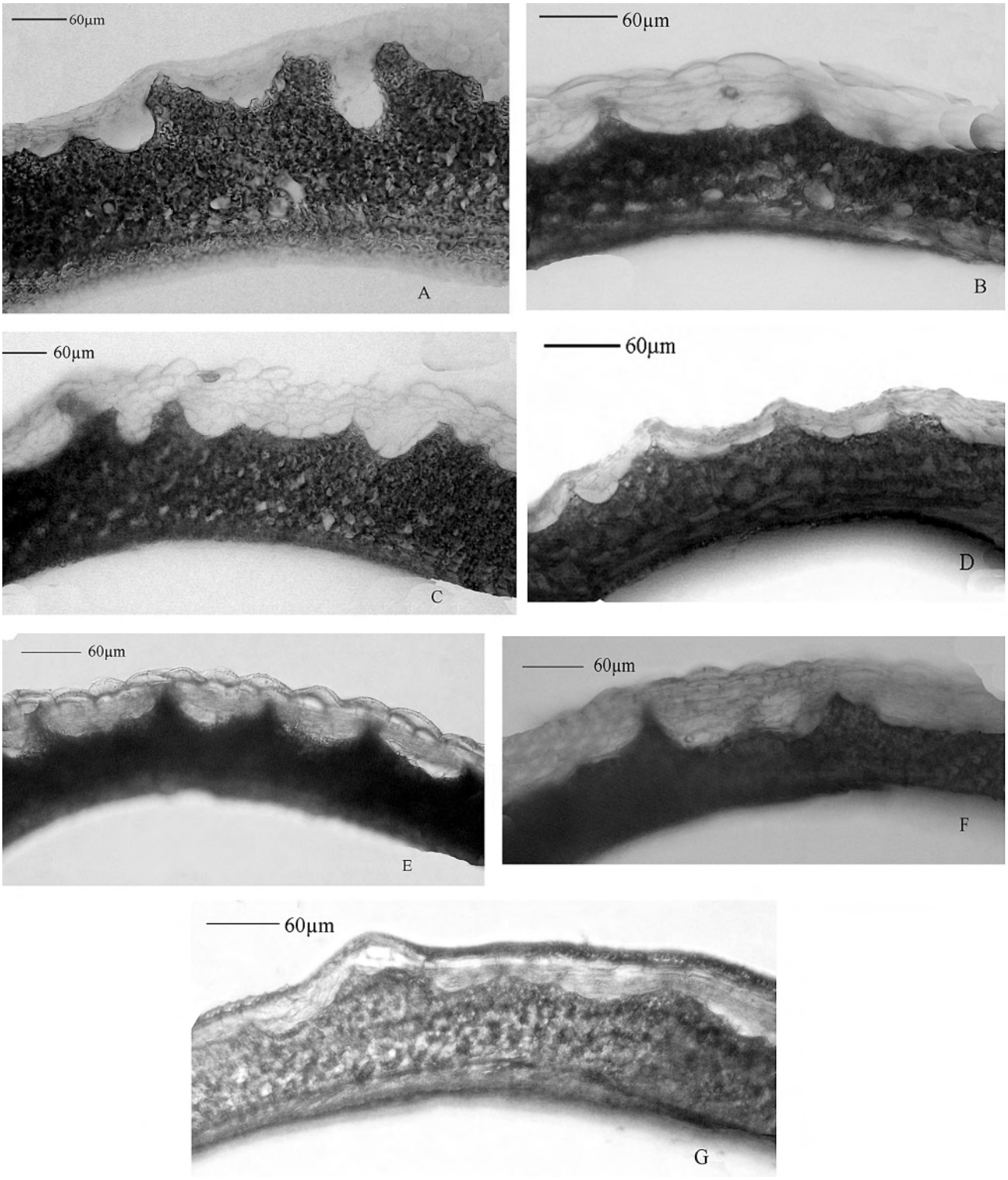

Fig. 5. Echinate ornamentation of the exocarp layer in the different species of *Fumaria*: **A,** *F. asepala*; **B,** *F. densiflora*; **C,** *F. indica*; **D,** *F. officinalis*; **E,** *F. parviflora*; **F,** *F. scelcheri*; **G,** *F. vaillantii*.

Table 4. Comparative results of fruit anatomical features in studied *Fumaria* species.

Taxon	Studied features					Endocarp shape under fruit apex
	Fruit Cross section shape	Sclerenchyma Thickness	Detailed echinate ornamentations	Fruit wing	Fruit apex shape	
F. asepala	Round to elliptic	228 µm	Sclerenchyma surface thick and rugose with short spine and acute apex	With 2 symmetric wings	Shallow notch	Crescent like
F. densiflora	Round	163 µm	Spine short, tapering and dense with obtous apex, recurve or branch	With 2 almost symmetric wings	Tall and acute apex	Crescent like which is not narrowed into margins
F. parviplora	Round to elliptic	95 µm	Without spine or short spine, acute apex and sparse spine	With 2 asymmetric wings	Short and obtuse apex	Crescent like which is narrowed into margins
F. vaillantii	Round to elliptic	81 µm	Spine short regularly distributed, acute or recurve ones sparsely	With 2 asymmetric wings	Very short and acute apex	Crescent like and very thin
F. indica	Round	67 µm	Very shortly spine with acute or obtuse apex distributed sparsely	Wing of fruit in one side and very thin	Very short and thin with obtous apex	Finely crescent like which is narrowed into margins
F. officinalis	Reniform or heart shape	75 µm	Sclerenchyma thick and rugose without short spine	One sided large crescent -shped wing	Short and acute apex	Dome shaped which is narrowed into margins
F. schleicheri	Round	61 µm	Sclerenchyma thin with shallow spine	With 2 almost Symmetric wings	Mucronate	not observed

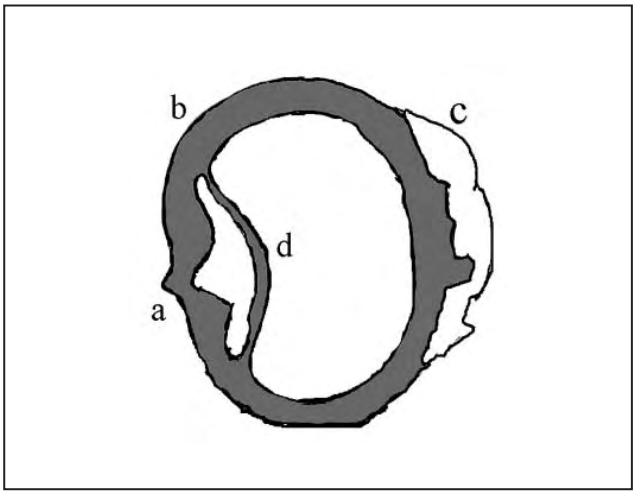

Fig. 4. Fruit cross section of *F. officinalis,* with reniform or cordate fruit shape. Legend: **a** – fruit apex, **b** – mericarp, **c** – wing, **d** – endocarp developed under the apical region.

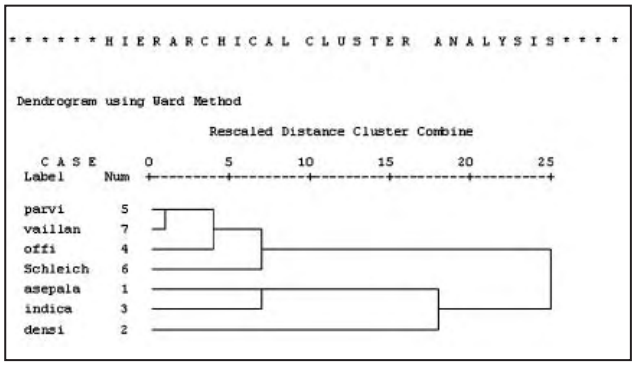

Fig. 6. A Ward's dendrogram of some *Fumaria* species in Iran

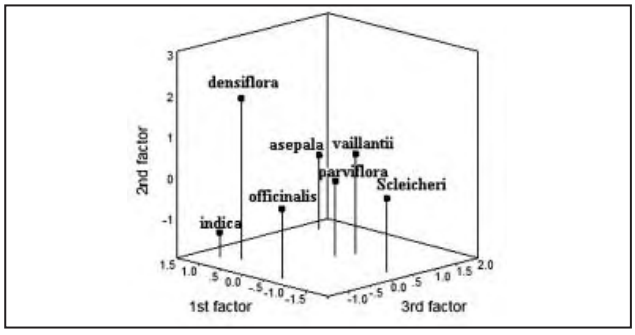

Fig. 7. PCA graph of some *Fumaria* species in Iran.

Discussion

The *Fumaria* species show a polyploid series, with numbers ranging from 2× to 14×, except 12×. In *Fumaria,* 46 out of 50 species are polyploid. Large-flowered species show a higher ploidy level, although the native species of Iran are small-flowered. Most species have showed polyploidy, and only four out of fifty are diploid (Lidén 1986). Diploids are not widely distributed species in Iran (*F. asepala*), in contrast to some of the polyploids, which are distributed in different habitats of Iran. As *F. parviflora* and *F. vaillantii* are recorded to be tetraploid (2n=32), it seems that the high range of anatomical variation in these taxa could follow from their ploidy level. A hexaploid species like *F. indica* (2n=48) is restricted to small areas and holds a separate position in the anatomical studies.

Anatomical observation of stem and root cross sections has revealed that symmetry, presence or absence of angles in the cross section and xylem shape are of diagnostic importance. Anatomical results of the species separation are in concordance with the earlier morphological results (Ebrahimzadeh 2008). Our results are in concordance with Kiliç & al. (2006), who has found a difference in the number of vascular bundles in the different species.

The results of the anatomical study of fruits have revealed that some anatomical features can be used to separate some species with high morphological similarities, such as *F. vaillantii*, *F. parviflora* and *F. indica*. *F. vaillantii* and *parviflora* share a lot of morphological characters, and have similar flavonoid patterns (Lidén 1991). Judging by the ordination of anatomical studies, *F. densiflora* is not close to *F. officinalis* as Lidén (1991) suggested. Our results are in concordance with those of the micro-morphological studies by Ebrahimzadeh (2008) of fruit and seeds in the *Fumaria* species of Iran.

References

APG III (Angiosperms Phylogeny Group). 2009. An update of the Angiosperms Phylogeny Group classification for the orders and families of flowering plants: APG III. – Bot. J. Linn. Soc., **161**: 105-121.

Budantsev, A.L. & Lobova, T.A. 1997. Fruit morphology, anatomy and taxonomy of tribe *Nepeteae* (Labiatae). – Edinb. J. Bot., **54**(2): 183-216.

Brückner, C. 1992. Gynoecium ontogeny and carpology in *Corydalis* DC. section *Cheilanthifoliae* Lidén (*Fumariaceae*). – Flora, **187**: 299-316.

Ebrahimzadeh, F. 2008. Biosystematic study of *Fumaria* species (*Papaveraceae*) in Iran. MSc Thesis. Biology Dept. Alzahra Univ., Tehran, Iran (in Persian).

Fukuhara, T. 1992. Seed-coat anatomy of Japanese species of *Corydalis* and *Dicentra* (*Papaveraceae; Fumarioideae*). – Bot. Mag. Tokyo, **105**: 303-321.

Hidalgo, O. & Gleissberg, S. 2010. Evolution of the reproductive architecture in the Bleeding Hearts and Poppies (*Papaveraceae* s.l.). – Int. J. Plant Dev. Biol., **4**(S1): 76-85.

Ingrouille, M.J. 1986. The construction of cluster webs in numerical taxonomic investigation. – Taxon, **35**: 541-545.

Kiliç, S., Ince, A. & Özcelik, H. 2006. The anatomical characteristics of two economically important geophytes of Turkey: *Corydalis solida* subsp. *solida* and *Corydalis conorhiza* (*Fumariaceae*). – Ekoloji, **15**(60): 46-54.

Lidén, M. 1986. Synopsis of *Fumarioideae* (*Papaveraceae*) with a monograph of tribe *Fumarieae*. – Opera Bot., **88**: 1-133.

Lidén, M. 1991. Phenetics and *Fumaria* – a comment. – P1. Syst. Evol., **176**: 221-225.

Lidén, M. 2000. Notes on *Dionysia*, *Corydalis* and *Fumaria* in Iran. – Iran. J. Bot., **8**: 303-308.

Liu, M., Plunkett, G., Lowry, P.P. II, Van Wyk, B. & Tilney, P. 2006. The taxonomic value of fruit wing types in order *Apiales*. – Amer. J. Bot., **93**(9): 1357-1368.

Medikus, F.C. 1789. Philosophische Botanik I. Mannheim.

Miller, P. 1754. The Gardeners Dictionary. Abridged Ed. 4. London.

Pugsley, H.W. 1919. A revision of the genera *Fumaria* and *Rupicapnos*. – J. Linn. Soc. Bot., **44**: 233-354.

Soler, A. 1983. Revision of *Fumaria* species in Iberian Peninsula Iberica and Balearic Islands. – Lagascalia, **11**: 141-228 (in Spanish).

Souza., L., Oliveira Oyama, S. & Muneratto, J. 2008. Morphology and anatomy of the developing fruit of *Macfadyeana unguis-cati* (L.) A.H. Gentry *Bignoniaceae*. – Acta Bot. Venez., **31**(1): 1-14.

SPSS Inc. 1998. SPSS Base 9.0 for Windows User's Guide. SPSS Inc., Chicago IL.

Sukhorukov, A.P. 2007. Fruit anatomy and its taxonomic significance in *Corispermum* (*Corispermoideae, Chenopodiaceae*). – Willdenowia, **37**: 63-87.

Thorne, R.F. 1976. A phylogenetical classification of the Flowering plants. – Evol. Biol., **9**: 35-106.

Wendelbo, P. 1974. *Fumariaceae*. – In: **Rechinger, K.H.** (ed.), Flora Iranica. Vol. **110**, pp. 23-30. Akad. Druck- u. Verlagsanstalt, Graz.

Wilson, B.J., Daker, M.G. & Savidge, J.P. 1990. A phenetic comparison of some *Fumaria* spp. (*Fumariaceae*). – Pl. Syst. Evol., **172**: 51-63.