Bangladesh J. Bot. **46**(1): 93-101, 2017 (March)

ANATOMICAL STUDY ON *FRITILLARIA* SPECIES IN IRAN

FATEMEH NAMAZI[1], MAJID SHARIFI-TEHRANI[1*] AND LEILA SHABANI[1]

Department of Biology, University of Shahrekord, Shahrekord, Iran

Key words: Anatomy, *Fritillaria* species, Identification key

Abstract

Anatomy of 14 *Fritillaria* species including five endemics to Iran was investigated. Results showed variation in anatomical characters between species, which are used for numerical analysis to reveal relationships between the species, and for construction of an identification key. Results support the separation of *F. poluninii* as a distinct species, and the close relationship between subgenera *Theresia* and *Petilium*, and also between kotschyana and crassifolia groups.

Introduction

Fritillaria L. (Liliaceae) consists of about 100 species (165 taxa) broadly distributed over the northern hemisphere. Zagros Mt. chain of Iran is a center of diversity for the genus, where subgenera from Mediterranean, Caucasian, and SW Asian regions meet (Rix 1997). *Fritillaria* is represented in Iran with four subgenera and 18 species. Taxonomy of *Fritillaria* was reviewed several times (Baker 1874, Turrill and Sealy 1980, Rix *et al.* 2001) and the classification proposed by Rix *et al.* (2001) is supported, in its general lines, by the most recent phylogenetic studies (Ronsted *et al.* 2005, Day *et al.* 2014). According to these authors, the genus *Fritillaria* is monophyletic, but infra-generic phylogenetic relationships are unclear. Bakhshi-Khaniki and Persson (1997) studied the nectary morphology in south-western Asian *Fritillaria* species. Pollen morphology of the Turkish species was studied by Teksen *et al.* (2010). Zaharof (1988) studied the phenetic relationships between *Fritillaria* taxa in Greece based on vegetative and floral characters. The present study evaluated the anatomical features of stem, root and leaves in Iranian *Fritillaria* species to report on application of these characters in classification of Iranian representatives.

Materials and Methods

Plants were collected from their natural habitats during 2012-2013. Vouchers are deposited in the herbarium of Faculty of Science at Shahrekord University. Species, voucher number and the GPS coordinates (latitude, longitude) for localities include: *F. imperialis* L. [1268: (35.31708, 46.24309); 1269: (35.08343, 46.39673)]; *F. raddeana* Regel [1274: (37.43872, 56.67321); 1356: (37.19478, 57.38914)]; *F. persica* L. [1281: (37.29658, 45.16564); 578: (32.1298, 50.36539)]; *F. olivieri* Baker [Endem., 1151: (34.76569, 48.45485); 112: (35.60094, 46.94016)]; *F. reuteri* Boiss. [Endem., 1339: (32.16332, 50.79189); 1125: (37.48068, 45.01808)]; *F. straussii* Bornm. [1202: (35.21637, 46.28287); 1221: (35.08077, 46.39647)]; *F. crassifolia* Boiss. & A. Huet ssp. *kurdica* Rix [1129: (37.47939, 45.01433); 1172: (35.29321, 46.20245)]; *F. poluninii* (Rix) Bakhshi Khaniki & Persson [206: (35.20071, 46.28115); 1289: (35.28913, 46.22274)]; *F. assyriaca* Baker [1236: (35.28396, 47.11823); 173: (37.48059, 45.01337)]; *F. uva-vulpis* Rix [137: (35.21708, 46.29505); 139: (35.21588, 46.29469)]; *F. atrolineata* Bakhshi-Khaniki [Endem., 1244, 1245: (37.29324, 45.1688)]; *F. caucasica* Adams [1284, 1288: (38.38909, 46.87418)]; *F. chlorantha* Hausskn. & Bornm. [Endem., 1231, 1234: (36.00272, 45.93134)]; *F. zagrica* Stapf. [Endem., 59: (32.18671, 50.76292); 105: (35.60782, 46.92445)].

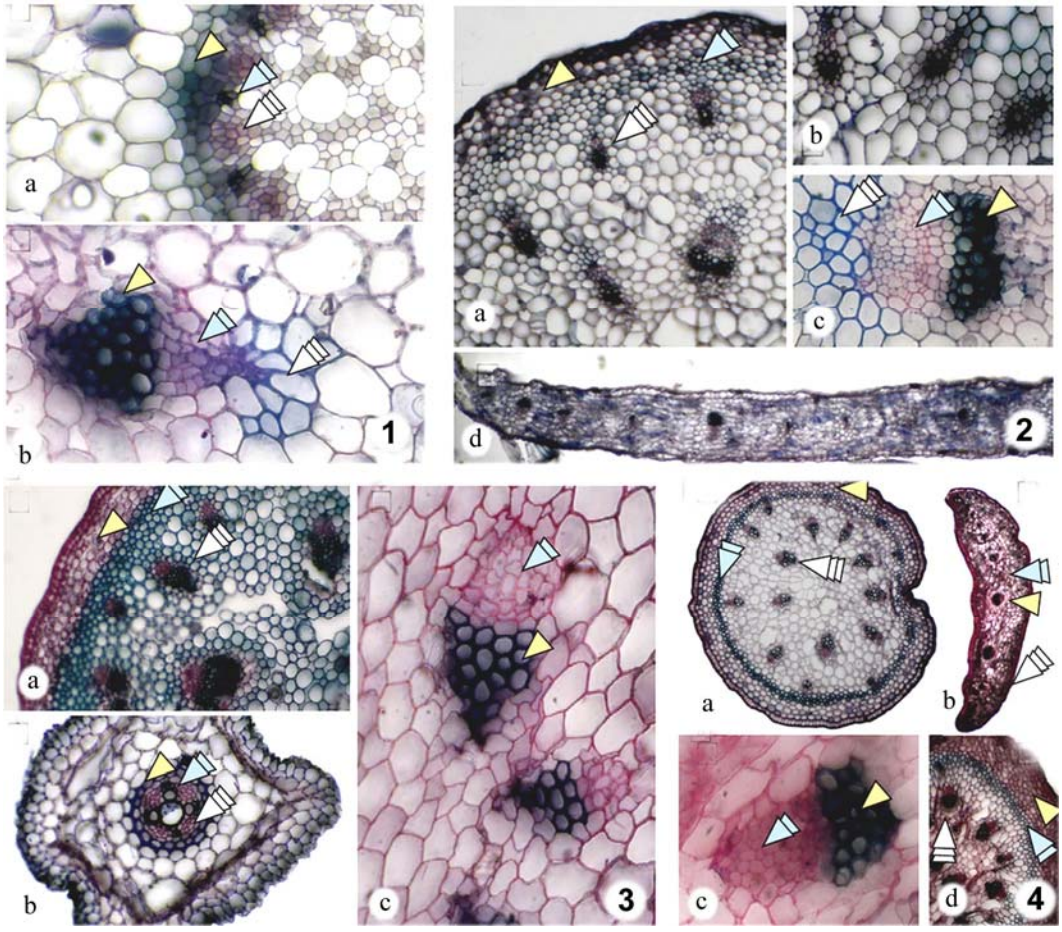
*Author for correspondence: <sharifi-m@sci.sku.ac.ir>. [1]Research Institute of Biotechnology, Shahrekord University, Shahrekord, Iran.

Identification of the specimens was made according to Rix (1997). Cross sections from the middle of stem, root and leaves at the late flowering period, were made by hand and double-stained with methyl green and Carmen stains. Good preparations were photographed using a Canon EOS 500D digital camera, mounted on an Olympus light microscope. Twenty nine qualitative anatomical characters were selected and studied on each specimen. Characters and their states were: 1: Stem CS shape (circular, elliptic, deltoid); 2: Stem surface (smooth, plicate, lobed); 3: Stem hypodermis rows (<4, >=4); 4: Lignified hypodermis rows (<2, >=2); 5: Ruptured hypodermis (Y/N); 6: Ruptured or air chamber in stem pith (Y/N); 7: Xylem/phloem ratio (>1, =1, <1); 8: Phloem bundle shape deltoid (Y/N); 9: Xylem pole shape deltoid (Y/N); 10: Xylem bundle shape adjacent to phloem is lunette (Y/N); Phloem fibers (Y/N); 12: Stem cortex cells thickened (Y/N); 13: Stem pith cells thickened (Y/N); 14: Root hypodermis cells smaller than cortex cells (Y/N); 15: Rupture between hypodermis and cortex (Y/N); 16: Root vascular bundles (=3, =4, >4); 17: Regular endodermis in root (Y/N); 18: Endodermis rows (=1, >1); 19: Root center hollow (Y/N); 20: Thick cuticle on epidermis (Y/N); 21: Root CS shape (circle, lobed, plicate); 22: Casparian strip (Y/N); 23: Casparian strip shape (lunette, throughout); 24: Root cortex with latex cells (Y/N); 25: Amilifer cells in cortex (Y/N); 26: Leaf surface furrowed (Y/N); 27: Midrib of leaf eminent (Y/N); Thick mesophyll in leaf (Y/N); 29: Thick cuticle in leaf (Y/N). Multi-state characters were converted to two-state (binary) characters (qualitative characters), and scores entered into a 0/1 matrix, which was used for comparisons between species and multivariate analysis using UN5 coefficient ($S_{UN5} = \frac{ad}{\sqrt{(a+b)(a+c)(b+d)(d+c)}}$) in NTSYSpc software package.

Results and Discussion

Stem anatomical features varied between *Fritillaria* species (Figs 1-14). Stem surface was smooth in subgenera *Petilium* and *Theresia*, and was plicate in *kotschyana* and *crassifolia* groups of sect. *Fritillaria* (except for *F. straussii*; a member of group *crassifolia* with lobed stem surface). Stem surface was smooth in members of group *caucasica* of sect. *Olostyleae*, except for endemics *F. zagrica* and *F. atrolineata* with plicate stem surfaces. Overall shape of stem in cross section was deltoid in *F. olivieri*; different from other species with elliptical to round shape in CS. Cross section of stem was uniformly round in members of *Petilium* and *Theresia*, but elliptical in members of group *crassifolia* (except for *F. straussii* which was round). Cross section of stem was also circular in all members of group *caucasica*, except for *F. zagrica* and *F. atrolineata*. The number of layers of hypodermis beneath the epidermis varied between species. *F. reuteri*, *F. assyriaca*, *F. uva-vulpis*, *F. atrolineata* and members of *Theresia* and *Petilium* markedly showed more than 4 rows of non-lignified cells. Number of lignified rows of cells in hypodermis adjacent to cortical cells, varied between species, but was constantly more than 3 rows of cells in *Petilium* and *Theresia*. Rupture between hypodermis lignified and non-lignified cell rows was observed only in two species; *F. straussii* and *F. zagrica*; other species showed an integrated hypodermis. Proportion of xylem and phloem elements in vascular bundles in stem varied between species. *F. raddeana* and *F. atrolineata* had more phloem elements per one vascular bundle compared to xylem elements; other species had equal or less phloem elements per vascular bundle in stem. Overall shape of xylem element bundle and the shape of tip of xylem bundle (away from corresponding phloem bundle) varied among species. Phloem fiber in stem was observed in *Petilium* (*F. imperialis* and *F. raddeana*), *F. crassifolia* and *F. chlorantha*. Cortical cells of thickened cell walls were observed in *Theresia* and *Petilium*. *Theresia* differed from *Petilium* in lacking pith cells of thickened cell walls. *Theresia* and *Petilium* are distinguished from each other and from *Crassifolia* based on stem anatomical features.

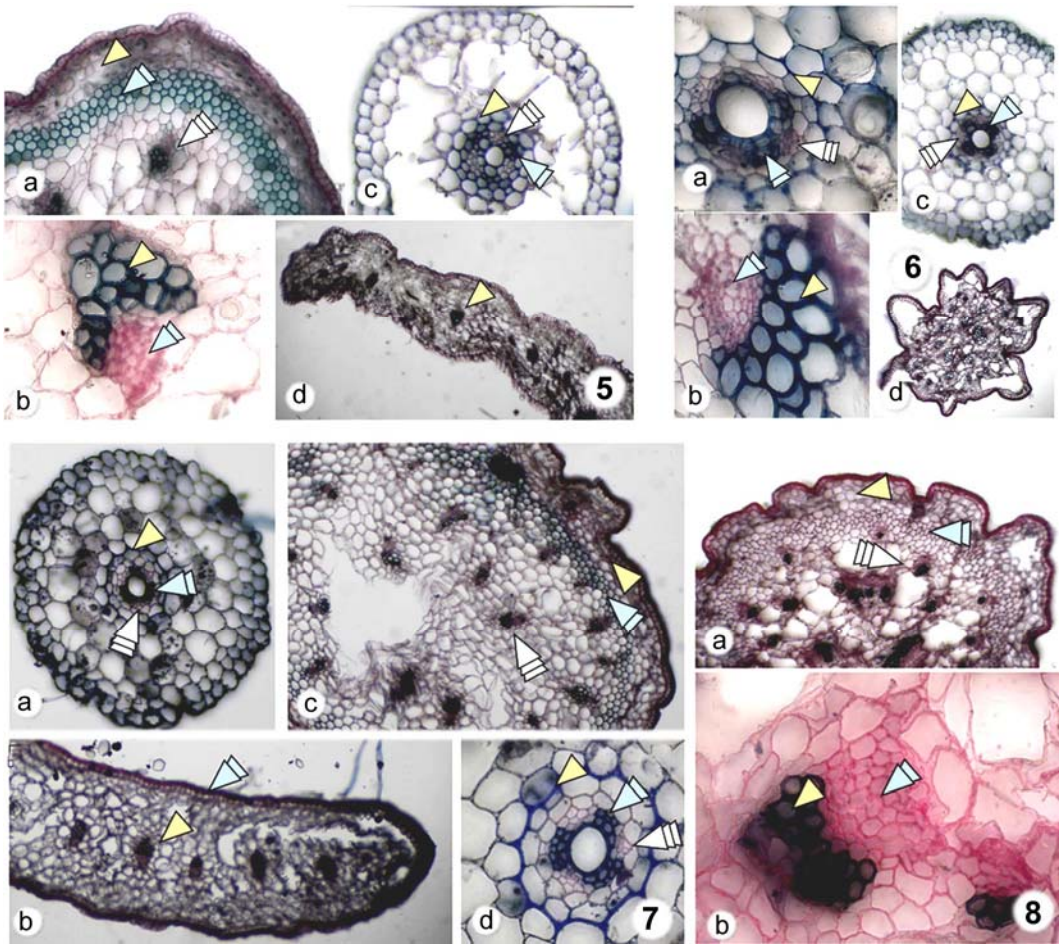
Root anatomical features also varied between species. *F. imperialis* differed from other species by having hypodermal cells markedly smaller than cortical cells. Rupture of cells between cortical and hypodermal cells was observed in all species except for *F. persica* and *F. reuteri*. *F. imperialis* contained several vascular bundles in vascular cylinder of root; *F. persica* and *F. straussii* had four vascular bundles and other species had three. Lacking of a regular endodermis,



Figs 1-4. CS of vegetative parts of *Fritillaria* species (light microscopy). 1. *F. imperialis*, a: root CS, b: stem vascular strand. 2. *F. raddeana*, a: stem CS, b: magnified stem CS, c: stem vascular strand, d: leaf CS. 3. *F. persica*; a: stem CS, b: root CS, c: stem vascular strand. 4. *F. atrolineata*; a: stem CS, b: stem vascular strand, c: leaf CS, d: magnified stem CS.

multi rows endodermis, lacking of Casparian strip, and existing of non-hollow root pith were diagnostic characters for subgenus *Petilium*. Lobed root shaped in CS is characteristic to subgenera *Petilium* and *Theresia*. Other species showed round root shape in CS, except for *F. poluninii* and *F. assyriaca* with plicate roots in CS. Casparian strip was absent in *Petilium*, was a complete ring in *Theresia* and was lunette in other species. Existence of amyllifer and latex cells in roots were variable.

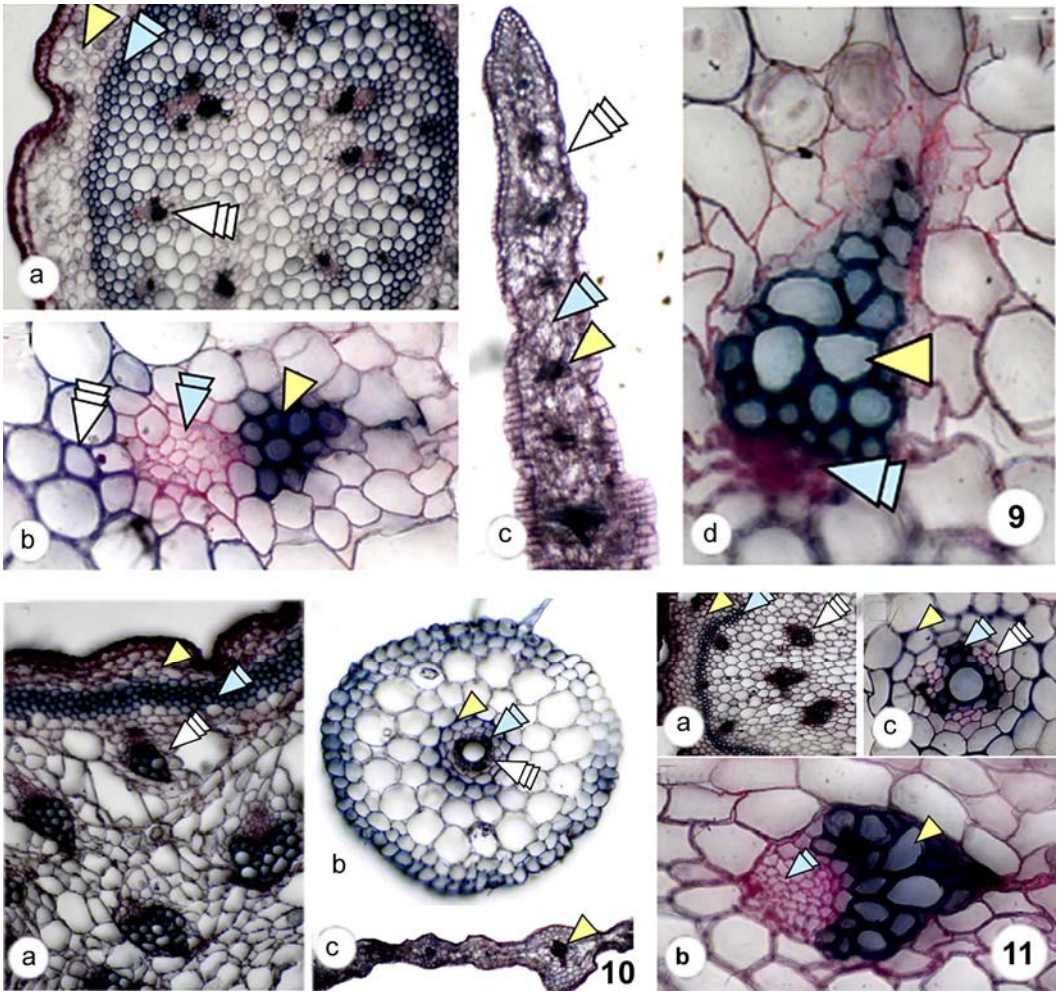
Leaf anatomical features varied between studied species (Figs 1-14). Leaf midrib was markedly raised in abaxial surface in *F. olivieri* and *F. imperialis*. Leaf surface was furrowed in different species with no relation to the taxonomy of species. Thickness of mesophyll and cuticles were also variously distributed in species. However, non-thick cuticles was shared by subgenera *Petilium* and *Theresia*.



Figs 5-8: 5. *F. reuteri*, a: stem CS, b: stem vascular strand, c: root CS, d: leaf CS. 6. *F. straussii*, a: magnified root CS, b: stem vascular strand, c: root CS, d: stem CS. 7. *F. crassifolia*; a: root CS, b: leaf CS, c: stem CS, d: magnified root CS. 8. *F. zagrica*, a: stem CS, b: stem vascular strand.

Multivariate analysis cumulated 55.13 per cent of variation in the first three axes (Fig. 15). Eigen value and percent of variation in first three axes were 2.29 (26.05%), 1.44 (16.33%) and 1.12 (12.75%), respectively. Plot of PCO analysis showed three major groups which was according to the classification of species at subgeneric and sectional levels. Group I consisted of subgenera *Theresia* and *Petilium*, group II of subgenus *Fritillaria* sect. *Fritillaria* (groups *kotschyana* and *crassifolia*), and group III of subgenus *Fritillaria* sect. *Olostyleae* (group

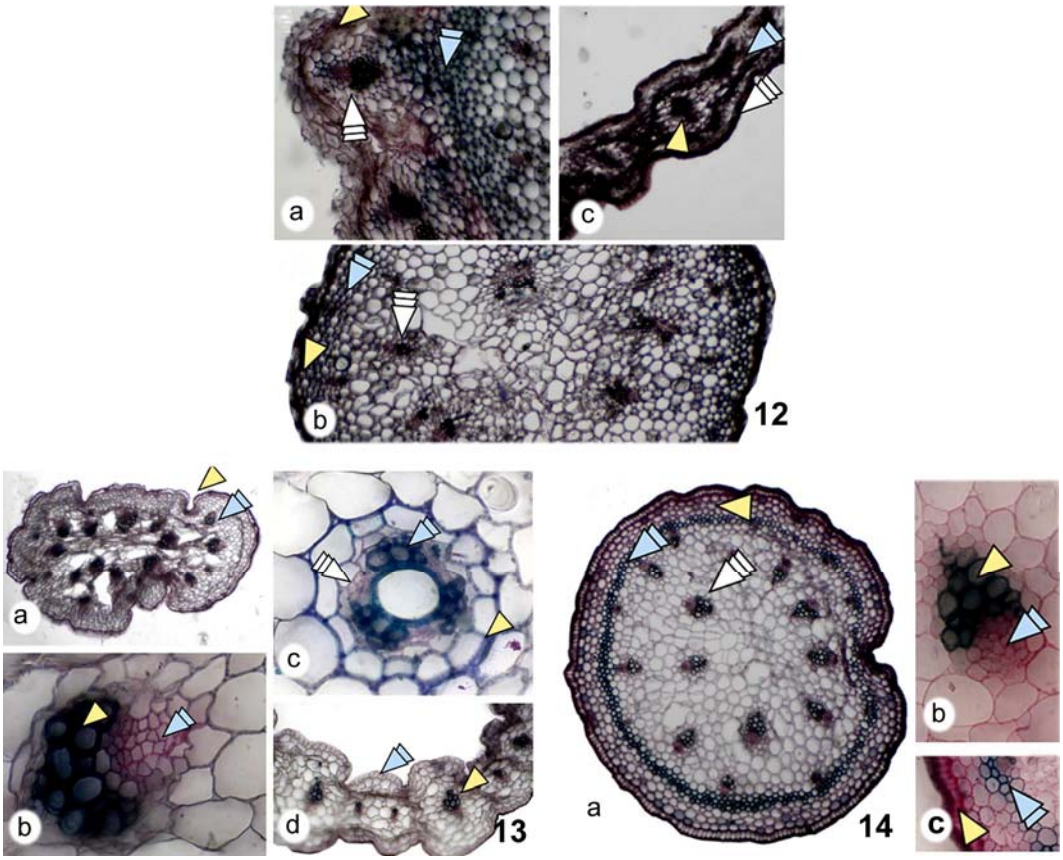
caucasica). The section *Fritillaria* (*F. olivieri* and the members of the group *crassifolia*), is the most represented taxon in group II, with *F. olivieri* situated between *F. straussii* and *F. assyriaca* and supports for the sectional classification. Close relationships between subgenera *Petilium* and *Theresia* is reported by recent studies (Turktas *et al.* 2012). Results of our study also showed their close phenetic relationships. Many shared anatomical characteristics support for close relationships between subgenera *Petilium* and *Theresia*. The subgenus *Fritillaria* consists of plants



Figs 9-11: 9. *F. uva-vulpis*, a: stem CS, b: stem vascular strand, c: leaf CS, d: stem vascular strand. 10. *F. olivieri*, a: stem CS, b: root CS, c: leaf CS. 11. *F. assyriaca*, a: stem CS, b: stem vascular strand, c: magnified root CS.

with a solitary or a few flowered racemose inflorescence, composed of regular narrow or wide campanulate flowers. Subgenus *Fritillaria* is non-monophyletic based on recent molecular studies (Ronsted *et al.* 2005, Day *et al.* 2014). The group *crassifolia*, which encompasses plants with linear nectaries and wide campanulate perigon, shared also a number of anatomical characters, such as, stem overall shape in CS, plicate surface of stem, rupture in stem pith, hypodermal cells

smaller that cortical cells, lunette Casparian strip, and root epidermis with thick cuticle. Corneanu and Popesco (1981) studied *F. imperialis*, *F. meleagris*, and *F. montana* in Romania and reported that stem anatomy could reveal differences among the species and may be used for their delimitation. They highlighted the two hypodermal tissue layers: a parenchyma zone outside and a sclerenchyma zone inside in which the size of layers, the number of cell rows, and the size and shape of the cells



Figs 12-14: 12. *F. chlorantha*, a: magnified stem CS, b: stem CS, c: leaf CS. 13. *F. poluninii*, a: stem CS, b: stem vascular strand, c: magnified root CS, d: leaf CS. 14. *F. caucasica*, a: stem CS, b: stem vascular strand, c: stem sclerenchyma beneath hypodermis.

For root CSs: arrow: endodermis, double arrow: xylem, triple arrow: phloem. For stem CSs: arrow: hypodermis, double arrow: sclerenchyma, triple arrow: vascular strand. For stem vascular strands: arrow: xylem, double arrow: phloem. For leaf CSs: arrow: vascular strand, double arrow: mesophyll, triple arrow: lower epidermis.

were characteristic of the species. In present study, these characteristics were important in separation of subgenera *Theresia* + *Petilium* (group I in Fig. 15) with 4 or more tires of parenchyma cells, from subgenus *Fritillaria* (cluster I + II) with most of taxa having less than four tires of parenchyma cells. Zaharof (1988) conducted a multivariate analysis on Greek *Fritillaria* species using morphological characters and evaluated the phenetic similarities among the species. The first three factors accounted for 64.5% of the variance, and it was claimed that the numerical

analysis could be useful in sketching certain phylogenetic and taxonomic conclusions. In our study, groupings were reproduced according to Rix *et al.* (2001) at subgeneric and sectional levels. It should be noted that the relationships among species within subgenus *Fritillaria* is still obscure, similar to the recent molecular phylogenetic studies (Ronsted *et al.* 2005, Turktas *et al.* 2012, Day *et al.* 2014). In his study on Greek fritillary species, Zaharof (1988) stated that "the occurrence of so many endemics each confined to its own island, mountain, or promontory, poses questions whether all these endemics merit taxonomic distinction at the rank of species".

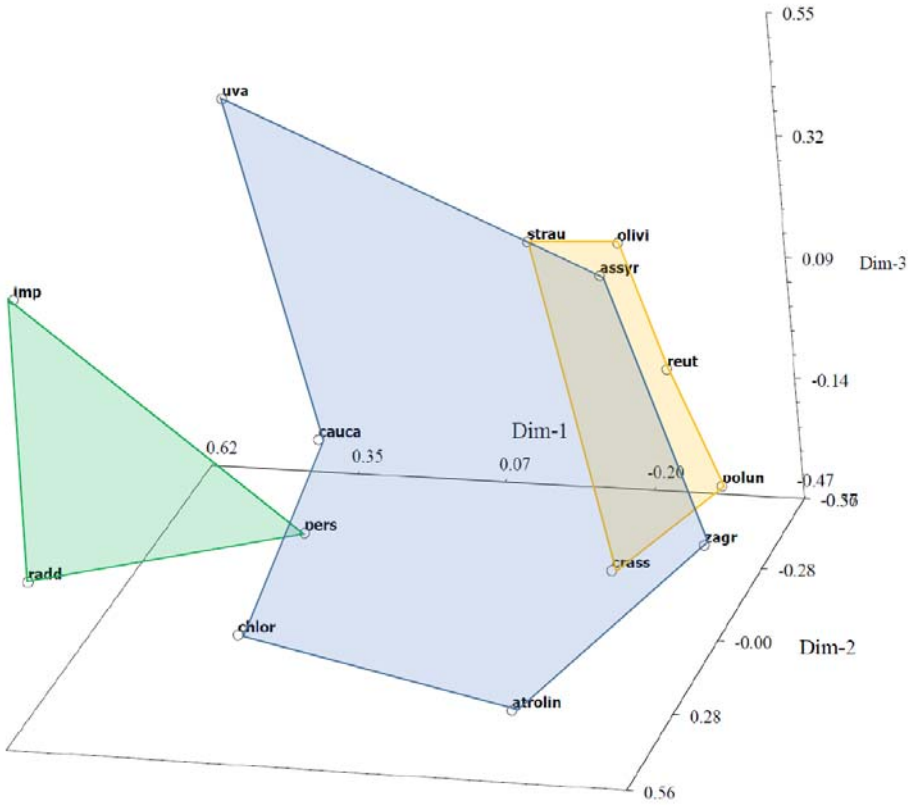


Fig. 15. Plot of PCO analysis of 41 qualitative anatomical characters measured on 14 species of *Fritillaria*.

Dichotomous key based on stem anatomical features.

1a-	Stem cortical cells thickened	
2a-	Phloem fibers present	
3a-	Phloem cells more than xylem cells in one vascular bundle	*F. raddeana*
3b-	Phloem cells not more than xylem cells in one vascular bundle	*F. imperialis*
2b-	Phloem fibers absent	*F. persica*
1b-	Stem cortical cell walls not thickened	
4a-	Stem CS shape deltoid	*F. olivieri*
4b-	Stem CS shape elliptical or rounded	
5a-	Stem surface lobed	*F. straussii*

5b-	Stem surface plicate or smooth	
6a-	Ruptured hypodermis between parenchyma and sclerenchyma	*F. zagrica*
6b-	Hypodermis not ruptured	
7a-	Phloem cells more than xylem cells in one vascular bundle	*F. atrolineata*
7b-	Phloem cells not more than xylem cells in one vascular bundle	
8a-	Stem shape in CS elliptical	
9a-	Hypodermis parenchyma 4 rows or more	*F. reuteri*
9b-	Hypodermis parenchyma less than 4 rows	
10a-	Phloem fiber absent, xylem cells more than phloem cells in one vascular bundle	*F. poluninii*
10b-	Phloem fiber present, xylem cells not more than phloem cells in one vascular bundle	*F. crassifolia*
8b-	Stem shape in CS rounded	
11a-	Phloem fiber present, hypodermis sclerenchyma 3 rows or more	*F. chlorantha*
11b-	Phloem fiber absent, hypodermis sclerenchyma 2 rows or less	
12a-	Stem surface plicate (furrowed)	*F. assyriaca*
12b-	Stem surface smooth	
13a-	Hypoderm parenchyma 4 rows or more	*F. uva-vulpis*
13b-	Hypoderm parenchyma less than 4 rows	*F. caucasica*

In the current study authors examined the usefulness of anatomical characteristics for classification of *Fritillaria* and results showed that anatomical characteristics provide useful diagnostic characters. Present results were concordant to the currently known classification of the genus. *Fritillaria poluninii* showed prominent differences from *F. crassifolia* specially in having phloem fibers and more xylem elements in stem vascular bundles. Section *Fritillaria* is supported by the close relationship of *F. olivieri* with members of group *crassifolia*. These results also revealed that monotypic *Theresia* was closely related to *Petilium*.

Acknowledgment

This research was supported by Deputy of Higher Educations and Deputy of Research at the Shahrekord University (Department of Biology, MS thesis No. 161.27 and Grant No. 94GRD1M873).

References

Baker JG 1874. A revision of the genera and species of Tulipeae. J. Linn. Soc. Bot. **14**: 211-310.

Bakhshi-Khaniki G and Persson K 1997. Nectary morphology in South West Asian *Fritillaria* (Liliaceae). Nord. J. Bot. **17**: 579-611.

Corneanu GC and Popesco GG 1981. Distributional and anatomical studies on *Fritillaria* (Liliaceae) in Romania. Willdenowia **11**: 307-315.

Day PD, Berger M, Hill L, Fay MF, Leitch AR, Leitch IJ and Kelly LJ 2014. Evolutionary relationships in the medicinally important genus *Fritillaria* L. (Liliaceae). Mol. Phylogenet. Evol. **80**: 11-19.

Rix EM 1997. *Fritillaria* L. (Liliaceae) in Iran. Iran. J. Bot. **1**: 75-95.

Rix EM, Frank E and Webster G 2001. *Fritillaria*: a revised classification, together with an updated list of species. *Fritillaria* Group of Alpine Garden Soc., Edinburgh.

Ronsted N, Law S, Thornton H, Fay MF and Chase MW 2005. Molecular phylogenetic evidence for the monophyly of *Fritillaria* and *Lilium* (Liliaceae; Liliales) and the infrageneric classification of *Fritillaria*. Mol. Phylogenet. Evol. **35**: 509-527.

Teksen M, Aytac Z and Pinar NM 2010. Pollen morphology of the genus *Fritillaria* L. (Liliaceae) in Turkey. Turk. J. Bot. **34**: 397-416.

Turktas M, Aslay M, Kaya E and Ertugrul F 2012. Molecular characterization of phylogenetic relationships in *Fritillaria* species inferred from chloroplast *trn*L-*trn*F sequences. Turk. J. Biol. **36**: 552-560.

Turrill WB and Sealy JR 1980. Studies in the genus *Fritillaria* (Liliaceae). Hookers Icones Plantarum **39**: 1-2.

Zaharof E 1988. A phenetic study of *Fritillaria* (Liliaceae) in Greece. Plant Syst. Evol. **161**: 23-34.

(Manuscript received on 9 August, 2015; revised on 7 September, 2016)