BIOSYSTEMATIC STUDY OF FUMARIA L. (PAPAVERACEAE) SPECIES IN IRAN

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Fumaria (*Papaveraceae*) comprises 8 annual species with medicinal and weed importance in Iran. Numerical study of 40 accessions of 6 species of this genus in Iran was studied by using 38 qualitative and quantitative morphological features. Banding patterns of seed storage proteins using sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with extract of bulked seeds of some *Fumaria* species of Iran as: *F. parviflora*, *F. vaillantii*, *F. asepala* & *F. indica* has been studied. The results showed that despite morphological observations the two very similar and sympatric species, *F. parviflora* and *F. vaillantii,* are not closely related due to their SDS profiles. *F. vaillantii* showed more similarity in seed storage proteins to *F. asepala* and *F. indica*. The intermediate position for *F. indica* is in concordant with its proposed progenitor as: *F. asepala* and *F. parviflora*. Electophoretic results are compared with morphological studies.

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Key words. SDS-Page, Fumaria, Iran, Relationships.

مطالعه بیوسیستماتیک گونه‌های جنس Fumaria L. (Fumariaceae) در ایران

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Fumaria از تیره شقایق مشتمل بر ۸ گونه یکساله با کاربردهای دارویی واهمیت علف هرز در ایران است. تاکسونومی عددی ٤٠ جمعیت از ٦ گونه از این جنس در ایران با استفاده از ۳۸ صفت کمی و کیفی ریختی مورد ارزیابی واقع شد. الگوی نواربندی پروتئینهای ذخیره‌ای بذر با استفاده از الکتروفورز SDS- Page با پروتین ذخیره بذر گونه‌های شاتره در ایران شامل *F. parviflora*, *F. vaillantii*, *F. asepala* و *F. indica* بررسی شده است. نتایج نشان می‌دهند که علی‌رغم شباهتهای ریختی مشاهده شده در دو گونه هم بوم *F. parviflora* و *F. vaillantii* این دو گونه ارتباط نزدیکی را با یکدیگر از نظر پروفیلهای پروتئین ذخیره بذر نشان نمی‌دهند. گونه *F. vaillantii* در پروتین ذخیره بذر بیشتر شبیه به گونه‌های *F. asepala* و *F. indica* است. وضعیت حد واسط مشاهده شده در گونه *F. indica* با اجداد پیشنهادی این گونه به صورت *F. asepala* و *F. parviflora* تطابق دارد. نتایج حاصل از الکتروفورز پروتئین ذخیره بذر با نتایج مطالعات ریخت شناسی مقایسه شده است.

INTRODUCTION

The genus *Fumaria* L. belongs to the *Fumarioideae* of *Papaveraceae*. In Flora Iranica 7 *Fumaria* species have been reported from Iran (Wendelbo 1974). However, Lidén has recently added *F. officinalis* L. as a new species for the flora of Iran (Lidén 2000). The earlier studies of the genus are restricted to Wendelbo 1974 and a very short report of Lidén from Golestan

Tabel 1. Voucher details of studied populations. (*Stands for sampled population in SDS Page studies).

Species	Vouchers
Fumaria vaillantii Loisel.	Mazandaran, Abeask, Ebrahimzadeh, 8610, AUH; Veresk, Ebrahimzadeh, 8611, AUH Veresk, Ebrahimzadeh, 8611, AUH; Gadok, Keshavarzi, 8612, AUH*; Karaj to chalus, Pole zanguleh 2400m, Ebrahimzadeh, 8613, AUH. Guilan, Southern Daylaman, 1305m, Nataj, 8617, AUH. Tehran, Jajrod, Ebrahimzadeh, 8614, AUH; Vanak, Alzahra university, Ebrahimzadeh, 8615, AUH; Abali, Mobarak abad, Nataj, 8616, AUH; Baraghan, Keshavarzi, 8620, AUH; Moderiat, Ebrahimzadeh, 8626, AUH*; Karaj, Mohamadshahr, Keshavarzi, 8633, AUH. Hamedan, Heidareh, Keshavarzi, 8628, AUH*; Ganjnameh, Keshavarzi, 8629, AUH; Kermanshah,Ostandari park, Gholami, 8630, AUH. Kordestan, Sanandaj, Abidar, Jahandideh, 8631, AUH. Fars,35 km to Marvdasht, Pasargard, Rastipisheh, 8634, AUH.
Fumaria parviflora Lam.	Tehran, Vanak, Alzahra university, Ebrahimzadeh, 8636, AUH Darband, 2200 m, Ebrahimzadeh, 8637, AUH; Karaj, Mohamadshahr, Keshavarzi, 8638, AUH; Abali, Mobarakabad, Nataj, 8639, AUH; Darabad, Rastipisheh, 8640, AUH. Golestan, Golestan national park, Ebrahimzadeh, 8641, AUH*. Lorestan, Dasht chegene, Direkvandi, 8642. AUH*; Garesan, Direkvandi, 8643, AUH; Fars, Fassa, Rastipisheh, 8646, AUH. Yazd, yazd, Keshavarzi, 8647, AUH; Taft, Keshavarzi, 8648, AUH*. Kermanshah, Ostandari park, Gholami, 8649, AUH.
Fumaria indica (Hausskn.) Pugsley	Tehran, Chitgar park, Ebrahimzadeh, 8635, AUH*.
Fumaria asepala Boiss.	Markazi, 22 km Saveh, 1290 m, Bolorian, 8651, AUH. Charmahal Bakhtiyari, Borojen, Yazdanbakhsh, 8652, AUH*. Gandoman, Yazdanbakhsh, 8653, AUH* . Fars, Kakan, Yazdanbakhsh, 8654, AUH. Tehran, Firouzkoh, Aminabad, Keshavarzi, 8655, AUH*; Darabad, Rastipisheh, 8656, AUH; Taleghan, Falatori, 8657, AUH; 100 Km Tehran- Saveh, 8658, AUH.
Fumaria densiflora DC.	Mazandaran, Kelardasht, Ebrahimzadeh, 8659, AUH; Namakabrod, Ebrahimzadeh, 8660, AUH; Galougah heights, Nataj, 8661, AUH.

National Park. There is no other systematic research in Iran. Lidén 1986 has prepared the monograph of the tribe *Fumarieae* but there is no collection from our region. Wilson et al. (1990) studied 11 species of this genus phenetically. They found a high rate of variation in *Fumaria* populations and also gene flow was recorded for some species. Identification key of *Fumaria* species in Flora Iranica is mainly based on quantitative features. Due to the high rate of variation in such characters in different soil types and habitats, it seemed inefficient. The present article considers numerical taxonomy and seed storage protein electrophoresis of the genus trying to indicate the species relationships. Moreover, diagnostic values of characters were checked statistically. As there is no integrated biosystematic study on the genus *Fumaria* in Iran, this paper aims to evaluate some Iranian *Fumaria species* using a phenetic approach based on the morphological and SDS- PAGE data.

METHODS & MATERIALS

Plant Materials

The voucher details of 40 sampled populations of 6 species are given in table 1. Voucher specimens, gathered from field are deposited at Herbarium of the Alzahra University (AUH). Specimens were gathered from nature. Characters selection was based on literatures (Murphy 2009), (Wendelbo 1974) and our own field observations. For each population 10 individuals were studied for qualitative and quantitative morphological characters. For numerical analysis, 38 qualitative and quantitative morphological characters were studied (tables 2 & 3).

Protein Extraction & Electrophoresis

In this study, ten populations were chosen for studying electrophoretic patterns of seed storage proteins. Seed samples were obtained from the sources indicated in table 1 with asterisk. From each accession, 1 gr. of seed was used. The final extract was loaded on SDS-PAGE and stained by comassic brilliant blue (Lammeli 1970). Standard Proteins (β galactosisase, Ovalbumin, Lactate dehydrogenase, lactoglobulin - β, Lysozyme & Bovine serum albumin) were used to evaluate the molecular weight of unknown proteins. The protein density was determined by Bradford Protocol. Banding patterns were studied and R.F. values were measured.

Tabel 2. Studied Quantitative morphological characters in *Fumaria* species of Iran.

Characters	unit
Leaf segment length	mm
Number of flowers per raceme	
Inflorescence length	mm
Pedicle Length	mm
Peduncle length	mm
Sepal length	mm
Sepal width	mm
Corolla Length	mm
Keel width	mm
Wing width	mm
Spur length	mm
Raceme length	mm

Tabel 3. Studied Qualitative and allometric morphological characters in *Fumaria* species of Iran.

Characters	State of characters
Leaf segment width (mm)	.2-.5 (0), .5-.7 (1), .7-1(2), 1< (3)
Stem direction	Erect (0), Diffuse (1)
Leaf segment shape	Linear (0), Linear- oblong (1), Lanceolate (2)
Peduncle	Absent (0), Nerly absent (1), Present (2)
Bract tip color	White (0), Violet (1)
Bract tip	Acuminate (0), Cuspidate (1)
Sepal presence	Yes (0), No (1)
Sepal shape	Ovate (0), Orbicular (1), Not present (2)
Sepal tip	Acuminate (0), Acute (1), Not present (2)
Sepal margin	Denticulate (0), Laciniate (1)
Sepal color	White (0), Pink (1), Violet (2)
Corolla color	White (0), Pale violet (1), Dark violate (2), Pink (3)
Spot color of inner petal	Purple (0), Black (1), Green (2), Greenish- Purple (3)
Outer petal tip	Emarginated (0), truncate (1), rounded (2)
Spot color of outer petal	Green (0), Greenish- Purple (1), Violate (2)
Lower petal tip	Emarginate (0), truncate (1), rounded(2)
Lower petal shape	Spathulate (0), Not so (1)
Fruit shape	Spherical (0), Cordate (1)
Fruit tip	Apiculate (0), Not so (1)
Fruit keel	Prominent (0), Not prominent (1)
Sepal width to sepal length	
Sepal width to corolla width	<1 (1), >1 (0)
Sepal length to corolla length	
Pedicel length to corolla length	
Spur length to spur depth	=1 (0), >1 (1)
Bract length to pedicle length	<1 (0), =1(1), >1 (2)

Statistical Analysis

In order to detect significant differences in studied characters among populations of each species and also among different species, analysis of variance (ANOVA) followed by the least significant differences (LSD) tests were performed.

To reveal species relationships we used cluster analysis and principal component analysis (PCA) (Ingrouille 1986). For multivariate analysis the mean of quantitative characters were used while qualitative characters were coded as binary /multi-state characters. Standardized variables were used for multivariate statistical analysis. The average taxonomic distances and squared Euclidean distances were used as dissimilarity coefficient in cluster analysis of morphological data. In order to determine the most variable morphological characters among the studied species, factor analysis based on principal components analysis was performed. SPSS ver. 9 (1998) and NTSYS software were used for statistical analysis.

In order to group the species with similar protein bands, cluster analysis and single linkage methods were performed. For this purpose, RM (Relative Mobility) values of protein bands were estimated and proteins having similar RM were taken as similar. Each protein band was taken as a qualitative character and coded as 1 (present) versus 0 (absence). Jaccards similarity index was determined among species followed by cluster analysis (fig. 4). In order to determine the most variable protein bands among studied species, factor analysis based on PCA was performed.

RESULTS

Phenetic analysis

By studying morphological characters by cluster analysis methods (neighbor joining, UPGMA) it became evident that all studied species are clearly separated (figs. 1 & 2). *F. officinalis* is not so similar to the other species so made a separate cluster. *F. densiflora* is closer to *F. officinalis* than the other studied taxa. *F. asepala* accessions made a separate cluster beside *F. parviflora.* Jafri (1974) indicated that *F. indica* is a hybrid between *F. asepala* and *F. parviflora*. Both dendrograms showed these similarities. It seems that *F. vaillantii* has some locally adapted ecotypes.

In order to determine the most variable characters among the studied species, factor analysis based on PCA was performed revealing that the first three factors comprise more than 80% of total variation. In first factor with 51.7% of total variation, sepal length and width, corolla length, wing and keel width of upper petal, spur length, sepal to petal length, width to length of sepal, sepal width to petal, pedicle to petal length,

Table 4. Jaccard similarity index based on electrophoretic data of seed storage protein in *Fumaria* taxa native to Iran (1: vaillan: *F. vaillantii* (Modiriat), 2 (Gadok) & 3 (Heydareh), 4: parvifl: *F. parviflora* (Dasht chegene), 5 (Golestan Park) & 6 (Taft), 7: *F. indica*, 8: *F. asepala* 1: (Gandoman), 9 (Borojen) & 10 (Amin abad).

Proximity Matrix

	Jaccard Measure									
Case	1: vaillan	2: vaillan	3:vaillant	4: parvifl	5: parvifl	6:parviflo	7: indica	8:asepala 1	9:asepala 2	10:asepa la3
1: vaillan		.933	.933	.459	.500	.425	.579	.658	.553	.595
2: vaillan	.933		1.000	.444	.486	.410	.568	.649	.541	.583
3:vaillant	.933	1.000		.444	.486	.410	.568	.649	.541	.583
4: parvifl	.459	.444	.444		.700	.759	.543	.390	.432	.514
5: parvifl	.500	.486	.486	.700		.742	.390	.364	.366	.474
6:parviflo	.425	.410	.410	.759	.742		.425	.364	.366	.474
7: indica	.579	.568	.568	.543	.390	.425		.703	.639	.844
8:asepala1	.658	.649	.649	.390	.364	.364	.703		.722	.771
9:asepala2	.553	.541	.541	.432	.366	.366	.639	.722		.706
10:asepala3	.595	.583	.583	.514	.474	.474	.844	.771	.706	

This is a similarity matrix

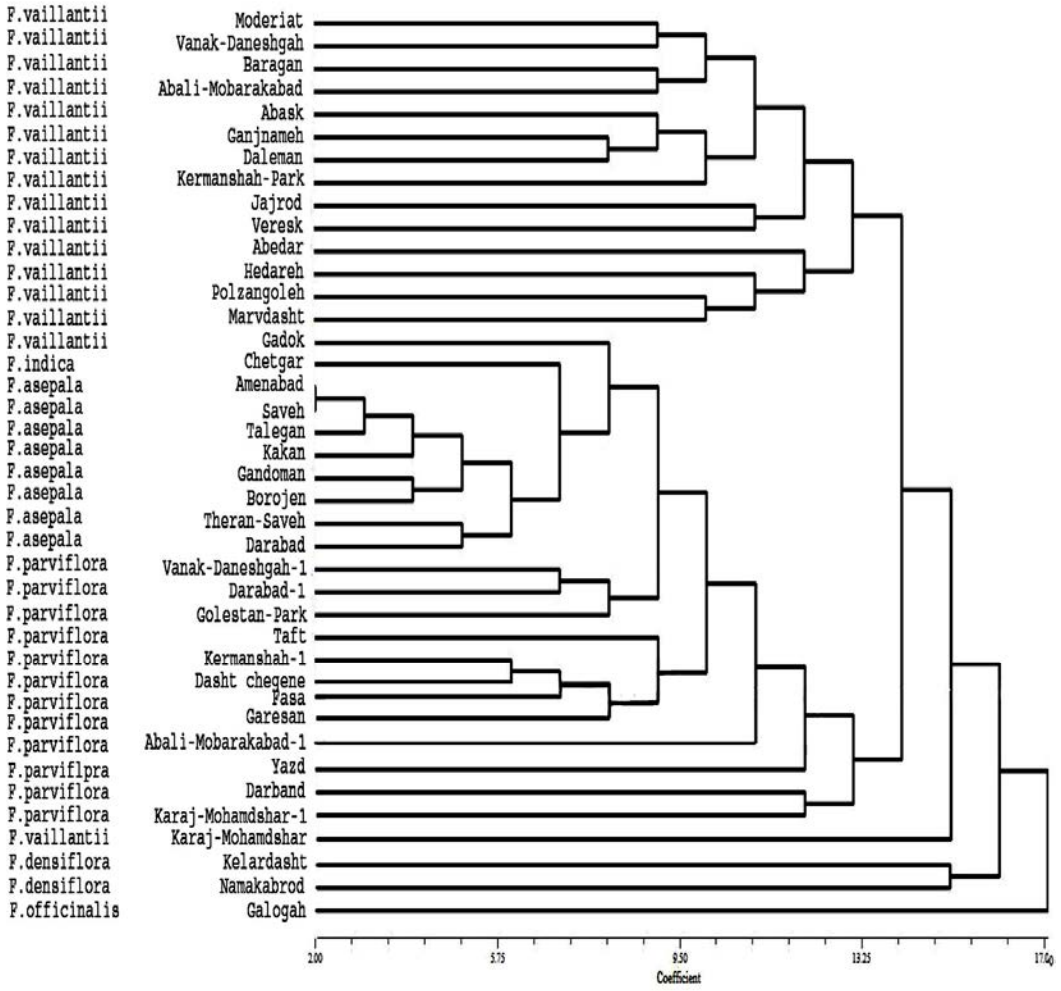

Fig. 1. Neighbor joining clustering of *Fumaria* species based on mean of quantitative morphological characters.

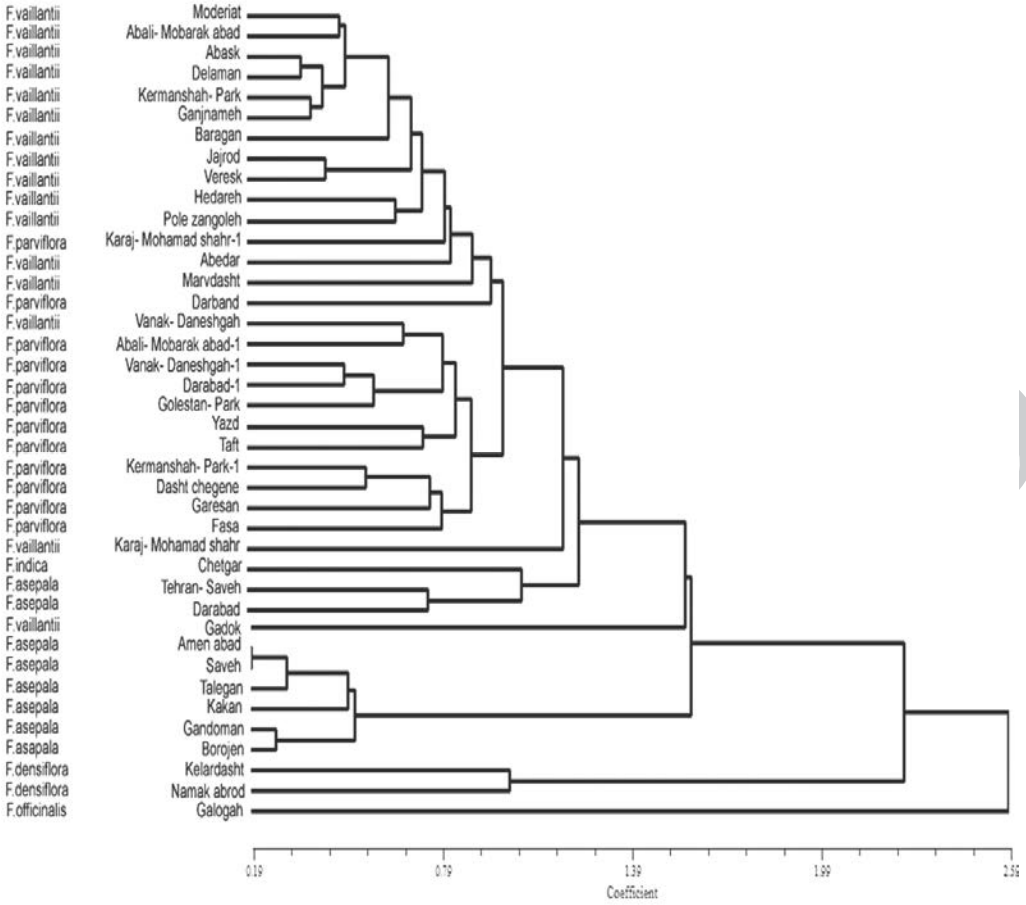

Fig. 2. UPGMA clustering of *Fumaria* species based on mean of quantitative morphological characters.

length to depth of spur, color spot on top of inner and upper petal, upper and inner petal top condition have the highest correlations. These features have clearly separated the *F. officinalis* from other studied species. In second factor with 19.3% of observed variation, presence, tip form and sepal margins have the highest correlation. In third factor with almost 10% of total variation, bract color has the highest correlations.

Ordination showed clear separation of *F. densiflora*, *F. officinalis* and *F. asepala* while there were some overlaps between different accessions of *F. vaillantii* and *F. parviflora* (fig. 3).

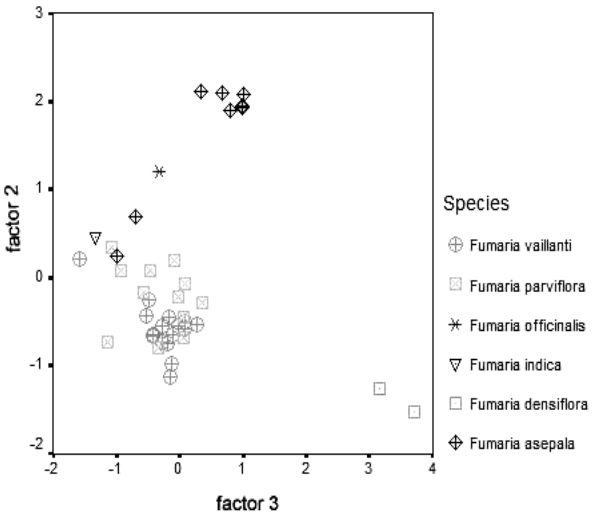

Fig. 3. PCA ordination of *Fumaria* species based on morphological characters.

SDS-Page profiles data

SDS-Page electrophoresis data were analyzed. Jaccard similarity index was evaluated (table 4). Totally 51 bands were observed for these taxa. The 11th, 15th, 17th, 22th, 30th, 33th, 34th, 35th, 43th, 47th and 51th bands were common in studied taxa. While band numbers 10, 23, 24 & 45 were only observed in *F. parviflora,* Bands number 13, 14, 16 & 29 were exclusively observed in *F. indica* and *F. asepala.* In *F. parviflora* from Taft the band numbers 8 & 27 and in Golestan population different bands as 26 & 39 were observed. Different population of *F. parviflora* showed some variations in

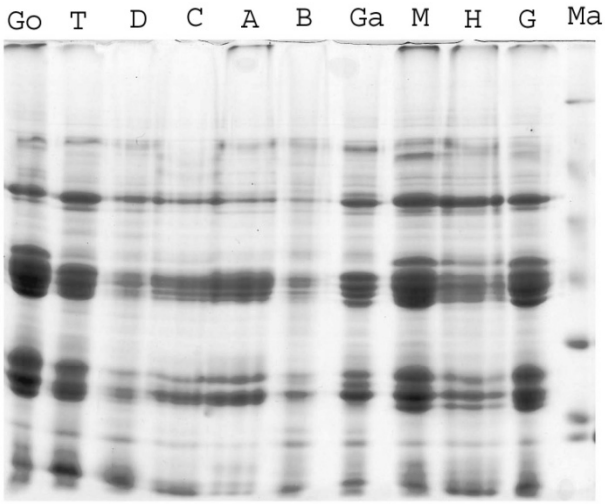

Fig. 4. The seed protein banding profile of wild *Fumaria* species native to Iran. GO) Golestan National Park, T) Taft & D) Dasht Chegeni populations of *F. Parviflora*, C) *F. indica*, A) Amin abad & B) Borojen & Ga) Gandoman populations of *F. asepala*, M) Modiriat, H) Heydareh & G) Gadok populations of *F. vaillantii*, Ma) marker.

their banding patterns. Highest numbers of bands was observed in *F. asepala* populations (fig. 4).

In order to find most variable protein band in studied taxa, Principal Component Analysis were done. Primitive analysis shows that 4 first factors are responsible for the 81% of total studied variation in taxa. In first factor with almost 38% of total variation, bands number 9, 25, 31, 32, 37, 40 & 42 have the highest positive correlations. In second factor with 23% of observed variation, band number 2 & 13, has the highest positive correlation. In third factor with almost 11% of total variation, bands number 7 and 12 have the highest positive correlations.

Cluster analysis result in dendrograms. We use both Neighbor Joining and UPGMA methods (figs 5 & 6). Taxa are clearly separated based on electrophoretic data of seed storage proteins. Results revealed that *F. indica* and *F. asepala* are closely related. High similarity index is a reflex of genomic identity (J= 0.857). *F. vaillantii* comprised a separate and distinct clade (fig. 5). Dendrogram shows close relationship and high protein similarity (J=0.583) between different populations of *F. parviflora*.

Neighbor joining and UPGMA dendrograms showed that there are some kinds of infra-specific grouping in *F. parviflora*. Studied taxa ordination, based on PCA (fig. 7), shows that there are in concordant with cluster analysis.

DISCUSSION

Morphological results of qualitative and quantitative features showed that some of the studied characters are of diagnostic importance. *Fumaria densiflora* by circular, cuspidate, dentate margins in sepal are clearly separated from other *Fumaria* species. *F. vaillantii* and *F. parviflora* have a close relationship which is in concordant with Ebrahimzadeh Araii et al. 2011. These species have some overlap in shape, margin, length, width and tip of sepals. These two can be separated by length to depth of spur and tip situation of upper petal. These species show similarities in pollen morphology too (Keshavarzi et al. 2011b).

Despite the results of morphological and anatomical observations (Keshavarzi et al. 2011a), UPGMA and NJ dendrograms of SDS- Page showed that the two very similar and sympatric species, *F. parviflora* and *F. vaillantii* are not closely related. *F. vaillantii* showed more similarity in seed storage proteins to *F. asepala* and *F. indica* populations. It is clearly evident that *F. parviflora* is separated by the SDS-Page electrophoresis data. As these three species are very mixed with each other and their proper identification is very difficult, it seems that SDS- Page provide a useful tool to separate these taxa form each other.

It is believed that *F. indica* is of hybrid origin between *F. asepala* and *F. parviflora*. In our results the similarity in seed storage proteins are observed between *F. asepala* and *F. indica* samples. As a final result it could be concluded that SDS-Page of seed storage protein is capable of providing diagnostic features in *Fumaria* species especially for separation of *F. vaillantii*, *F. indica* and *F. parviflora*. Different populations of *F. parviflora* showed some variations in their banding pattern and it seemed that they have made some subspecies in Iran. For recording *F. parviflora* probable subspecies, further studies are needed.

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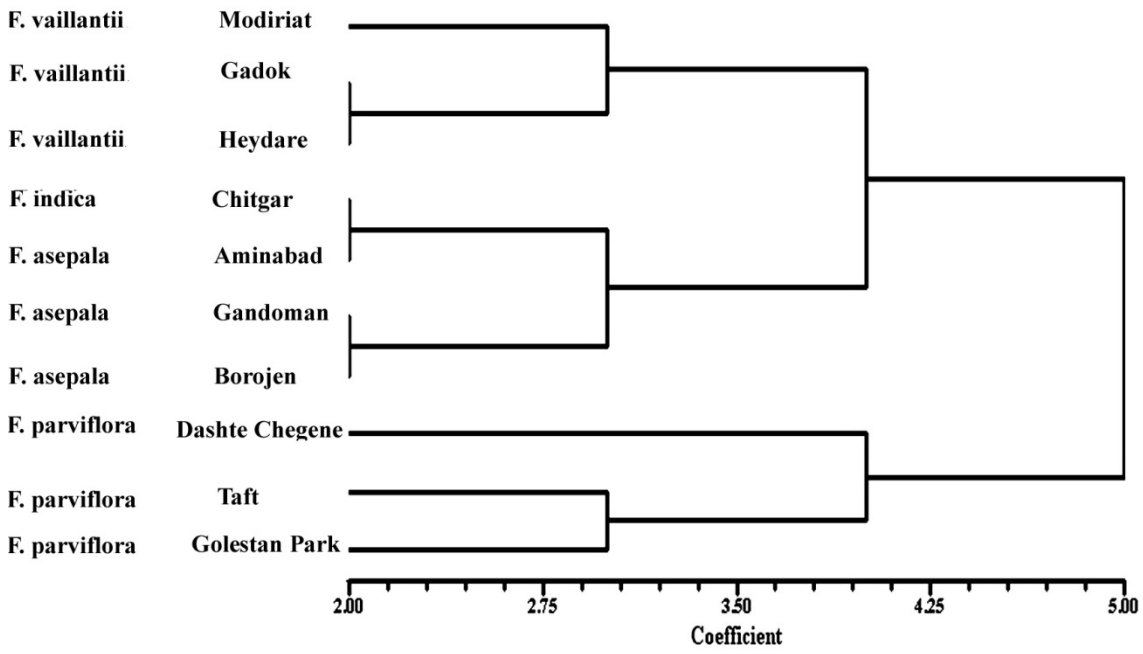

Fig. 5. Dendrogrum depicting clustering by Neighbor Joining Method of *Fumaria* species by cluster analysis of seed storage protein.

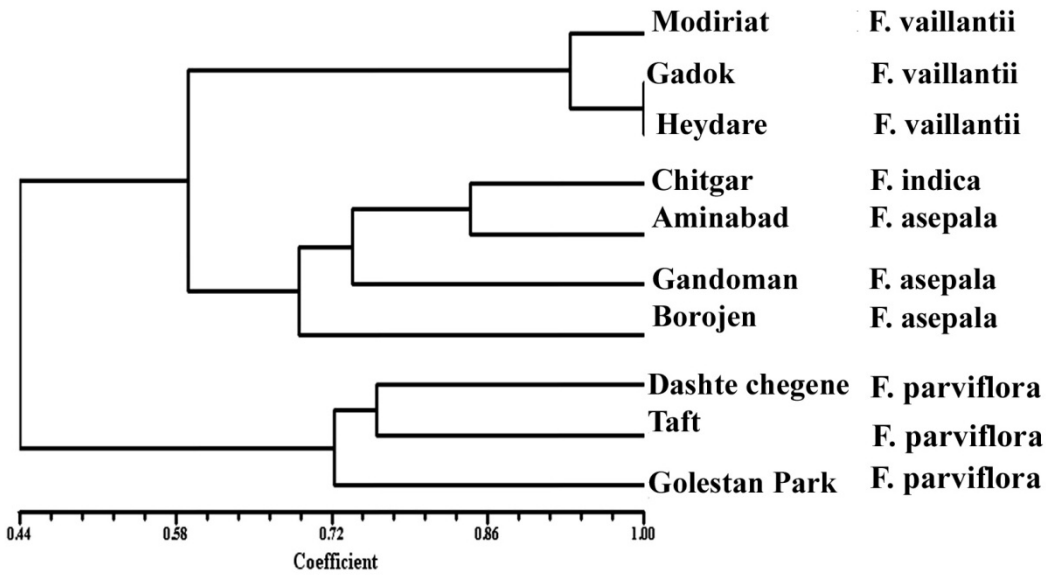

Fig. 6. Dendrogram of cluster analysis by UPGMA method based on seed electrophoresis results of *Fumaria* species native to Iran.

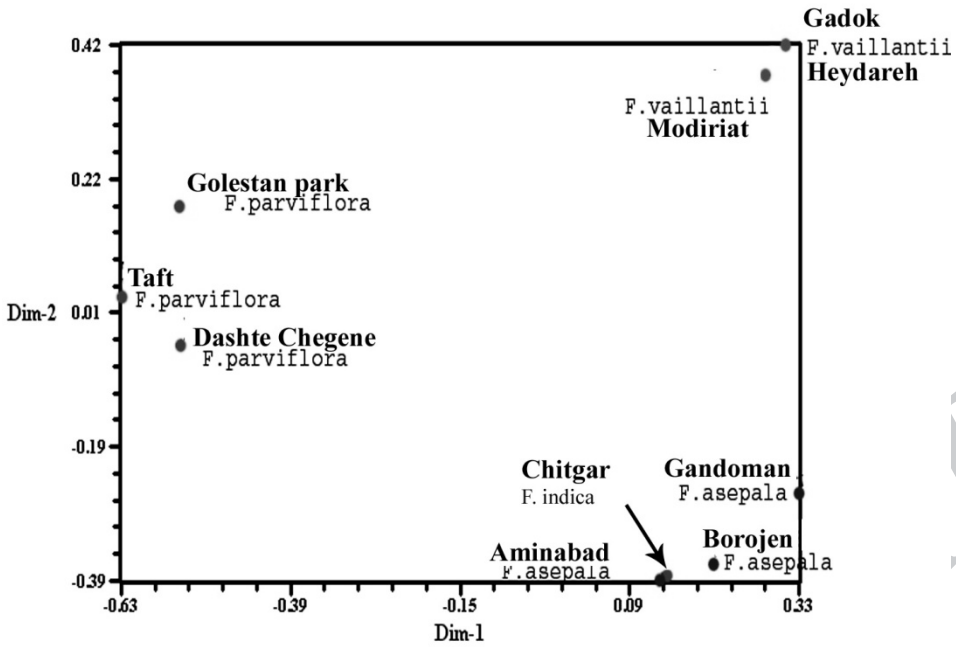

Fig. 7. PCO ordination of the *Fumaria* species based on SDS-PAGE characters.

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