

ARTICLE

Infrageneric classification of *Geranium* (Geraniaceae) based on fruit and seed morphology

Maryam Keshavarzi*

Faculty of Biological Sciences, Alzahra University, Vanak, Tehran, Iran

ABSTRACT *Geranium* comprises 23 to 25 annual and perennial species in Iran. Fruit and seed morphology of 9 taxa are examined to investigate utility of infrageneric system of *Geranium* in Iran. Totally 19 characters of fruits and seeds were evaluated by stereomicroscopy and scanning electron microscopy. Fruit and seed morphological markers are of evolutionary and taxonomic importance in *Geranium* species. Species relationships and infrageneric classification have been evaluated by multivariate statistical analysis. Diagnostic features are presence of glandular hairs in mericarp and hair length, seed width and mericarp texture. Taxa of two subgenera are clearly separated based on selected fruit and seed characters.

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KEY WORDS

Geranium
mericarp
micromorphology
scanning electron microscopy
seed

Introduction

Geranium L. (Geraniaceae) comprises 423 species in the world which are classified in 3 subgenera and 18 sections (Aedo et al. 1998; Mabberley 2008). The genus is represented in Iran by 23-25 species. These species are grouped in two subgenera and 8 sections (Schönbeck-Temesy 1970; Janighorban 2009; Onsoni et al. 2010). Diagnostic features in infrageneric classification are related to fruit discharge methods, mericarp margin and leaves shape. In Iran there are *Geranium* species with carpel projection or seed ejection.

The seed morphology of some *Geranium* species was examined by various workers (El Naggar 1992; Park and Park 2002; Ather et al. 2012). El Naggar (1992) investigated the seed morphology of 14 *Erodium* L Hér. species. He found that seed size, shape and surface pattern were of diagnostic importance.

Subgenus *Geranium* is the largest of the *Geranium* subgenera. From these subgenera only two are present in Iran. There is no species of *Erodioidea* (Picard) Yeo in Iran. Some *Geranium* species in Iran especially those in *Tuberosa* section are morphologically very similar and there are some intermediate between species.

Subgeneric classification is mainly based on the fruit discharge and morphology but there are no detailed reports on fruit and seed morphology of *Geranium* species of Iran. The present study provides fruit and seed morphological data of the representative species of each section of *Geranium*, as additional information for their taxonomic delimitation. Infrageneric classification will be evaluated by macro- and

micromorphological features of fruits and seeds.

Materials and Methods

Mature seeds of 9 *Geranium* species were collected from herbarium specimens (Table 1). Mostly, 10 individuals of each location were studied and examined for 19 qualitative and quantitative features (Table 2) under handheld digital stereomicroscope, Dino-Lite Pro (AM413T Model) and scanning electron microscope (SEM, JSM-6380A, JEOL).

For scanning electron microscopy dry seeds were directly mounted on metallic stub using double adhesive tape and coated with gold for a period of 6 minutes in sputtering chamber (BAL-TEC, SCDOOS) and observed under SEM. The terminology used is in accordance with previously published studies of Lawrence (1970), Radford et al. (1974) and Punt et al. (1994).

In order to detect significant differences in the studied characters among the various studied species, an analysis of variance (ANOVA) was performed. To reveal the species relationships, we have used cluster analysis and principal component analysis (PCA) (Ingrouille 1986). For multivariate analysis, the mean of the quantitative characters was used, while qualitative characters were coded as binary/multi-state characters. Standardized variables were used for multivariate statistical analysis. Average taxonomic distances and squared Euclidean distances were applied as dissimilarity coefficient in the cluster analysis of micromorphological data. In order to determine the most variable characters among the studied species, factor analysis based on principal components analysis was performed. SPSS ver. 19 software was used for statistical analysis.

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*Corresponding author. E-mail: keshavarzm@alzahra.ac.ir

Table 1. Voucher details of *Geranium* sampled in this study.

Species	Vouchers
<i>G. albanum</i> M. Bieb.	Mazandaran, Astaneh Ashrafiyeh, <i>Keshavarzi</i> , 91100 (HAU)* Golestan, Ziarat, <i>Pakravan</i> , 91110 (HAU)
<i>G. purpureum</i> Vill.	Golestan, Minodasht, Alghajar, <i>Habibi Tirtash</i> , 92100 (HAU) Gilan, Bandare Anzali, <i>Keshavarzi</i> , 92200 (HAU)
<i>G. columbinum</i> L.	Gilan, Langarud, <i>Keshavarzi</i> , 93100 (HAU)
<i>G. dissectum</i> L.	Gilan, Langarud, <i>Keshavarzi</i> , 94100 (HAU) Tehran, Khojir National Park, <i>Keshavarzi</i> , 94200 (HAU)
<i>G. lucidum</i> L.	Khuzestan, Izae, <i>Keshavarzi</i> , 95100 (HAU) Khuzestan, Dezful, <i>Keshavarzi</i> , 95200 (HAU)
<i>G. mascatense</i> Boiss.	Khuzestan, Dezful, <i>Keshavarzi</i> , 96100 (HAU)
<i>G. molle</i> L.	Golestan, Gorgan, Molaghati, <i>Keshavarzi</i> , 97100 (HAU) Mazandaran, Gaemshahr, Telar jungle, <i>Keshavarzi</i> , 97200 (HAU) Gilan, Lahijan, <i>Keshavarzi</i> , 97300 (HAU)
<i>G. tuberosum</i> L.	Mazandaran, Amol, <i>Keshavarzi</i> , 98100 (HAU) Tehran, Ahar, <i>Keshavarzi</i> , 98200 (HAU) East Azerbaijan, Marand, Yam, <i>Keshavarzi</i> , 98300 (HAU) Fars, Kazeroun, <i>Keshavarzi</i> , 90120 (HAU) Kurdistan, Bijar, <i>Keshavarzi</i> , 61100 (HAU)
<i>G. linearilobum</i> DC.	Kurdistan, Bijar, <i>Keshavarzi</i> , 91200 (HAU) Tehran, Narmak, <i>Keshavarzi</i> , 91201 (HAU)

*HAU: Herbarium of Alzahra University, Vanak, Tehran, Iran

Results

Mericarp of *Geranium* species are generally ellipsoid, globular or triangular and winged (Fig. 1). Texture differed from crispy to hard. As referred in the literature (Ather et al. 2012), generally, seeds are 0.8-3.8 x 0.6-2.2 mm in size, oblanceolate, oblong, subglobose, apex truncate, retuse or rounded, ridge present, brown, light brown, orange brown, reddish brown or blackish brown, undulate, foveate, rugosely foveate, foveately striate, rugosely striate, scalariform, areolate, areolate along with undulate, reticulate, reticulate foveate, reticulate with in reticulation foveate or appressedly reticulate, glabrous or sparsely pubescent (Table 3 and 4). Hilum is basal, sub basal or lateral (Fig. 1).

Fruit surface could be hairy, wrinkled or a combination of both types (Fig. 2, 3 and 4). There are some differences in length and width, shape, size, ornamentations and shape of *Geranium* seeds investigated. Seed surface ornamentations proved to be of diagnostic value in the studied species of Iran. There are both symmetrical and asymmetrical (*G. lucidum*) ornamentations on seed surface of studied species: reticulate and striate ornamentations are found to be the main types in these *Geranium* species (Fig. 5 and 6).

Table 2. Evaluated morphological characters (all quantitative features are in mm).

Feature	Scale / State of character	Code
Mericarp length	mm	M.L.
Mericarp width	mm	M.W.
Mericarp shape	0 - triangular 1 - elliptical 2 - rectangular 3 - suborbicular	M.S.
Mericarp surface ornamentation	0 - smooth 1 - wrinkled 2 - with prongs	M.S.O.
Mericarp hair length	mm	M.H.L.
Seed shape	0 - ellipsoid 1 - rectangular 2 - ovoid	S.S.
Seed color	0 - brown 1 - yellow 2 - other colors	S.C.
Seed surface ornamentation	0 - smooth 1 - reticulate	S.S.O.
Seed length	mm	S.L.
Seed width	mm	S.W.
Seed apex	mm	S.A.
Mericarp texture	0 - crispy 1 - thick	M.T.
Beak hair length	mm	B.H.L.
Wax in seed surface	0 - absent 1 - present	W.S.S.
Glandular hair in fruit surface	0 - absent 1 - present	G.H.F.
Hair on seed apex	0 - absent 1 - present	H.S.A.
Symmetry in seed ornamentation	0 - absent 1 - present	SY.S.
Attached hair to mericarp apex	0 - absent 1 - present	H.M.A.
Seed detailed ornamentations (SEM)	0 - striate 1 - reticulate 2 - cristate 3 - bireticulate 4 - glabrous 5 - undulate	S.D.O.

Statistical analysis by cluster analysis method (WARD) based on 19 qualitative and quantitative macro and micro-morphological characters of the fruits and seeds (Fig. 7) revealed the presence of 2 major clusters. *G. columbinum*, *G. dissectum*, *G. tuberosum* and *G. linearilobum* are grouped in the first cluster. These species are belonged to *Geranium* subgenus. The second cluster included *G. lucidum*, *G. molle*, *G. purpureum*, *G. albanum* and *G. maschatense*, which all belong to subgenus *Robertium*. In general, the closer connection between *G. columbinum* and *G. dissectum* is in concordant with their general morphology. PCA plotting based on fruit and seed characters (Fig. 8) also supports the results of the cluster analysis.

Table 3. Qualitative data matrix of the investigated species (abbreviations listed in Table 2)

Species name	Code											
	S.S.	S.C.	S.S.O.	W.S.S.	G.H.F.	H.S.A.	SY.S.	H.M.A.	M.S.O.	M.S.	S.D.O.	M.T.
<i>G. albanum</i>	1	3	1	1	2	1	1	1	2	0	1	1
<i>G. columbinum</i>	2	1	2	1	2	2	1	1	0	1	4	2
<i>G. dissectum</i>	2	1	2	1	2	2	1	1	0	1	4	2
<i>G. linearilobum</i>	1	1	2	2	2	2	1	1	0	1	2	2
<i>G. lucidum</i>	0	2	1	2	1	2	2	1	2	2	6	1
<i>G. maschatens</i>	1	1	2	2	1	2	1	1	2	3	2	1
<i>G. molle</i>	0	3	1	2	1	2	1	1	1	2	6	1
<i>G. purpureum</i>	0	2	1	2	1	2	1	2	1	1	1	1
<i>G. tuberosum</i>	0	1	2	1	2	2	1	1	1	1	2	2

Table 4. Quantitative data matrix of the investigated species (abbreviations listed in Table 2).

Species name	Code						
	M.L.	M.W.	M.H.L.	B.H.L.	S.L.	S.W.	S.A.
<i>G. albanum</i>	5.181	2.043	.500	.000	2.652	1.255	.000
<i>G. columbinum</i>	2.406	1.362	.360	.326	1.279	1.180	.000
<i>G. dissectum</i>	3.629	1.628	.422	.400	1.820	1.496	.000
<i>G. linearilobum</i>	4.167	1.358	.357	.346	2.247	1.355	.119
<i>G. lucidum</i>	2.220	1.190	.000	.000	1.630	1.006	.061
<i>G. maschatens</i>	5.095	2.361	.000	.000	2.630	1.003	.000
<i>G. molle</i>	1.641	1.164	.000	.000	1.450	.974	.000
<i>G. purpureum</i>	2.282	1.204	.000	.000	1.826	1.082	.000
<i>G. tuberosum</i>	3.600	1.784	.232	.329	2.141	1.324	.000

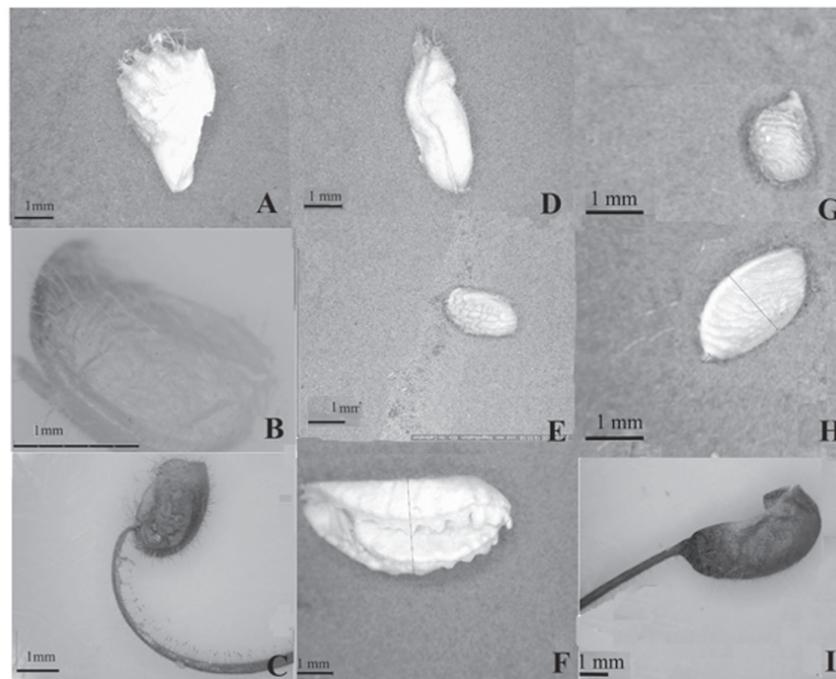


Figure 1. Stereomicroscopic micrographs of mericarp surfaces. A: *Geranium albanum*; B: *G. columbinum*; C: *G. dissectum*; D: *G. linearilobum*; E: *G. lucidum*; F: *G. maschatens*; G: *G. molle*; H: *G. purpureum*; I: *G. tuberosum*. Scale bar length is 1 mm.

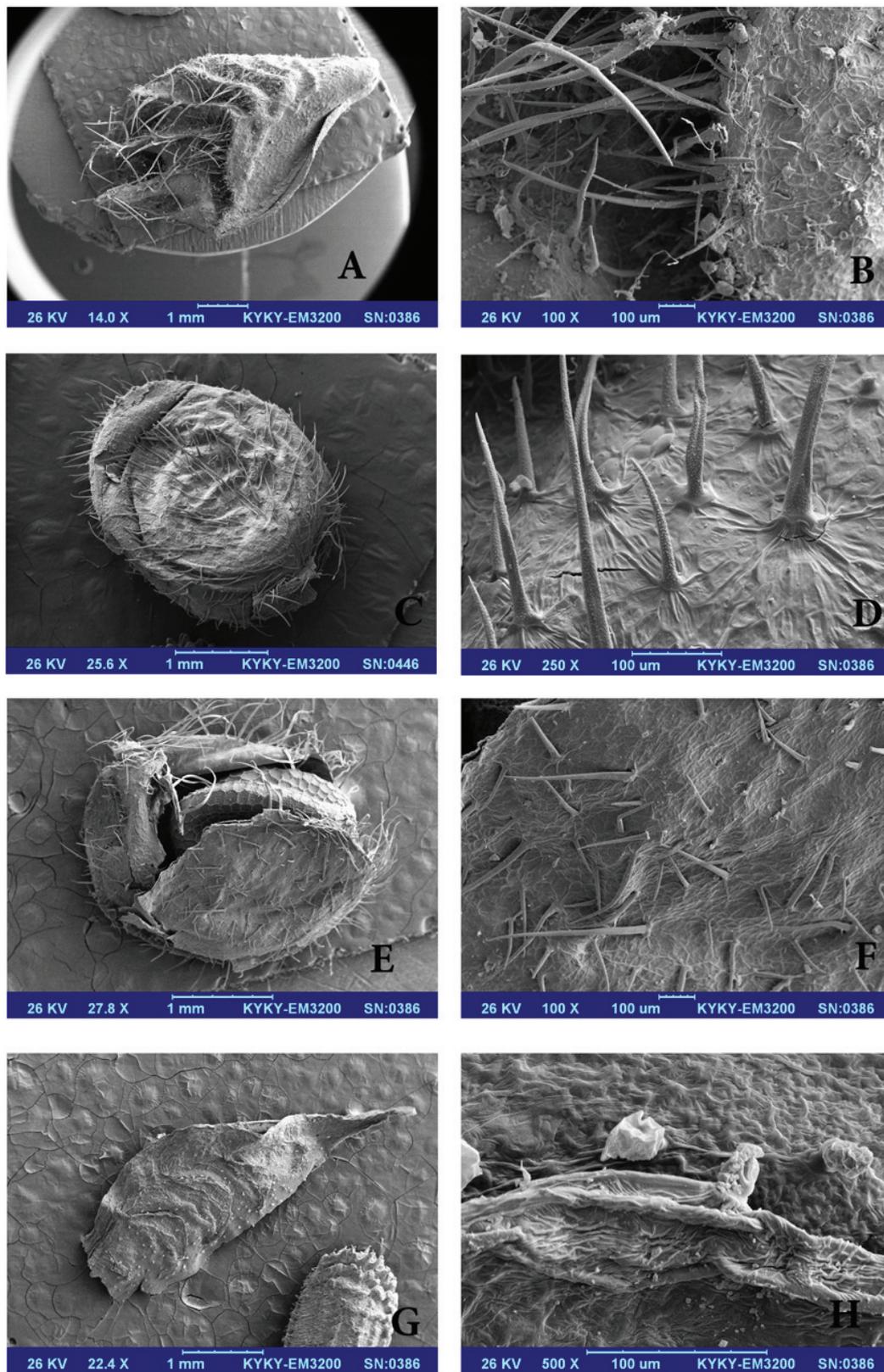


Figure 2. SEM micrographs of *Geranium* mericarps (shape and surface). A-B: *Geranium albanum*; C-D: *G. columbinum*; E-F: *G. dissectum*; G-H: *G. lucidum*. Scale bars are 1 mm and 100 µm for A, C, E, G and B, D, F, H, respectively.

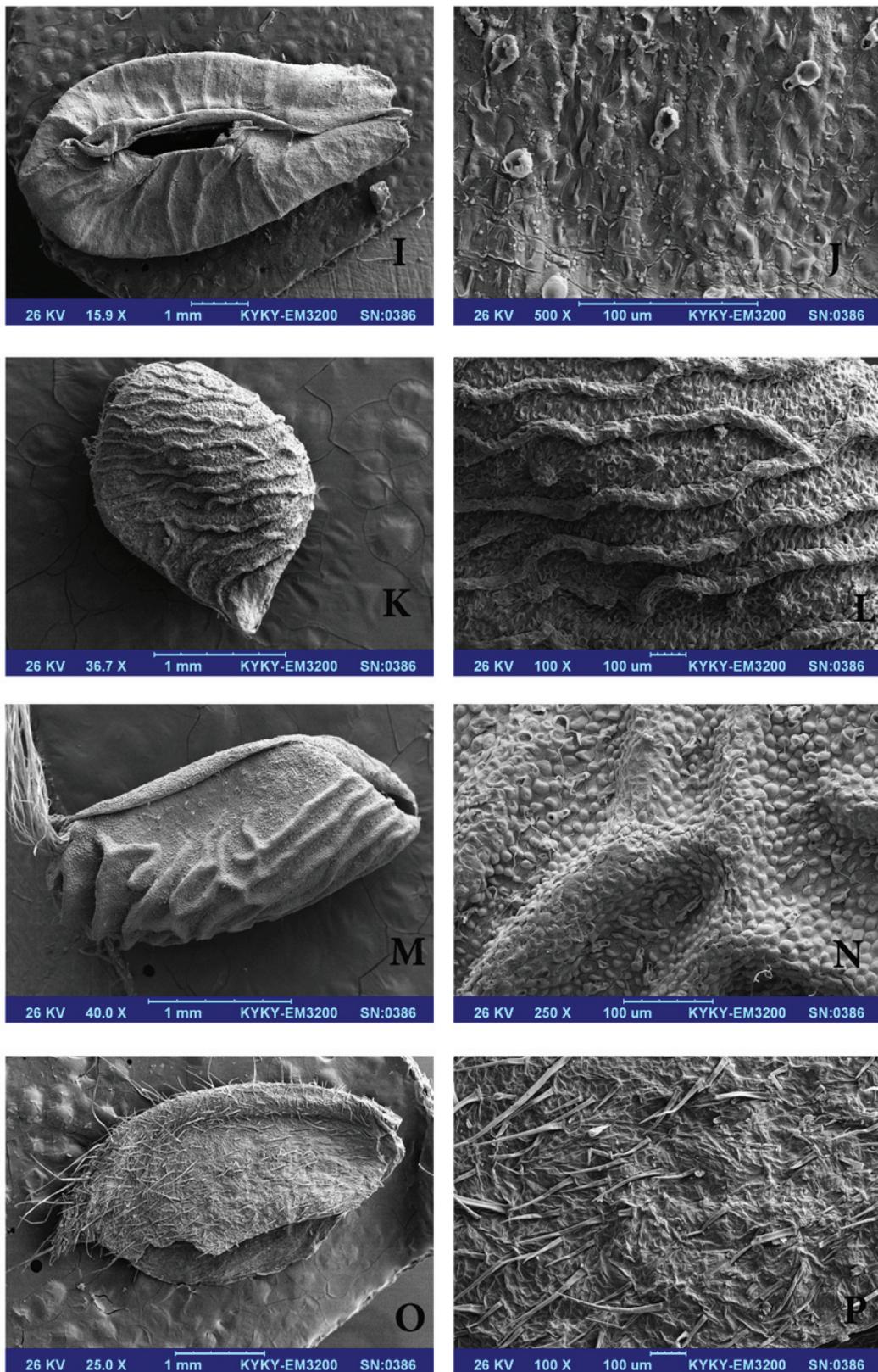


Figure 3. SEM micrographs of *Geranium* mericarps (shape and surface). I-J: *G. maschatens*; K-L: *G. molle*; M-N: *G. purpureum*; O-P: *G. lineari-lobum*. Scale bars are 1 mm and 100 µm for I, K, M, O and J, L, N, P, respectively.

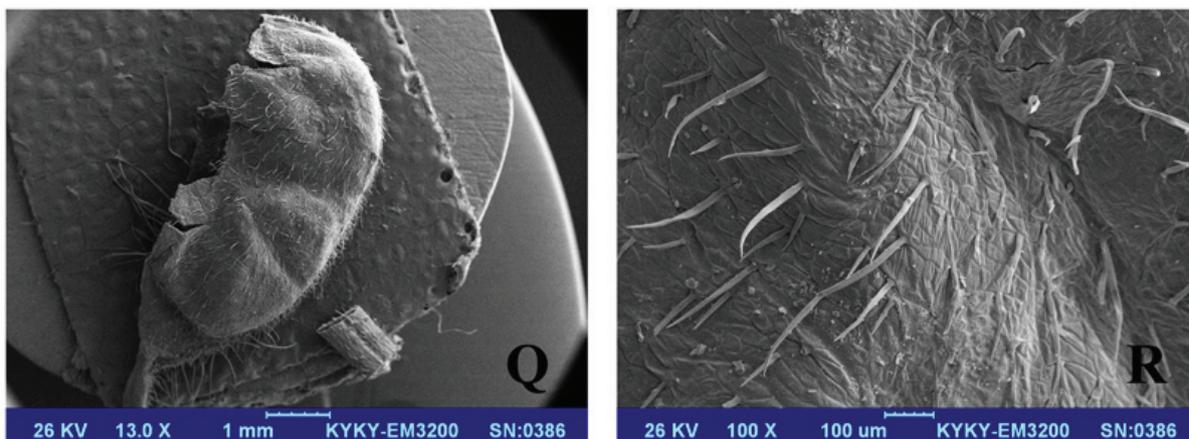


Figure 4. SEM micrographs of *Geranium* mericarps (shape and surface). Q-R: *Geranium tuberosum*. Scale bars are 1 mm and 100 µm for Q and R, respectively.

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 about 75% of total variation. In the first factor with more than 39% of total variation, characteristics such as glandular hair in seed surface, seed width, hair average length on mericarp and rostrum, seed sculpture, wax on seed and general seed shape possessed the highest correlation (≥ 0.7). In the second factor with about 22.4% of total variation seed length, hair at seed apex, length and width of mericarp possessed the highest correlations. In the third factor mericarp shape caused about 13.5% of total variation.

Based on these markers, the subgenera *Geranium* and *Robertium* are clearly separated (Fig. 8). There are more similarities between sections of *Geranium* subgenus. In subgenus *Robertium*, all sections are clearly related (except *G. albanum* species which has demonstrated an isolate position in PCA scatter diagram).

Discussion

Seed and fruit morphology is known to be proper source of diagnostic data for many plant taxa. In this study, morphology and micromorphology of fruits and seeds in selected *Geranium* species (forming part of the Iranian flora) were investigated to evaluate their potential for taxonomic separation of *Geranium* subgenera and sections.

The morphological and phylogenetic relationship of the taxa within *Geranium* species is well correlated with seed morphological data. Similarly, seed morphology is also found to be significant for infrageneric, specific or infra-specific

delimitations.

Janighorban (2009) used hair at leaf surface, pedicel length and hair type and presence of cilia at filaments base to separate *G. dissectum* and *G. columbinum*. Her study did not involve the investigation of fruit and seed diagnostic features. Our investigation has been carried out on a wider morphological base and found that her previous results are concordant with those of the results of the present project based on seed and fruit morphologies. At the same time, Butcher (1961) referred also to different characters of fruits and seeds. Among others, he pointed to wrinkled seed surface of *G. molle* and *G. lucidum*, or he mentioned hairy apex for *G. lucidum* too. Butcher (1961) used the same description for *G. columbinum* and *G. dissectum*: seed surface described as honeycombed.

Awn in subgenus *Geranium* sect. *Geranium* is permanent and helps a long distance dispersal of these seeds. At the same time, in subgenus *Robertium* awns are deciduous and seed dispersal is done in short distance in compare to the other subgenera in Iran. In this project, the value of seed and fruit morphology and micromorphology in infrageneric and sectional classification of *Geranium* in Iran has been studied for the first time and their diagnostic value has been proved.

When some species display a general morphological similarity, it is necessary to focus also on fine characters e.g., their fruit and seeds to have a better separation. Fruits and seeds features are also of crucial diagnostic value when the investigated specimen have not complete leaves and flower parts. Further studies on some related species as *G. divaricatum* Ehrh and *G. albanum*, and *G. robertianum* L. and *G. purpureum* could clarify the value of fruit and seed morphology and micromorphology also in these taxa.

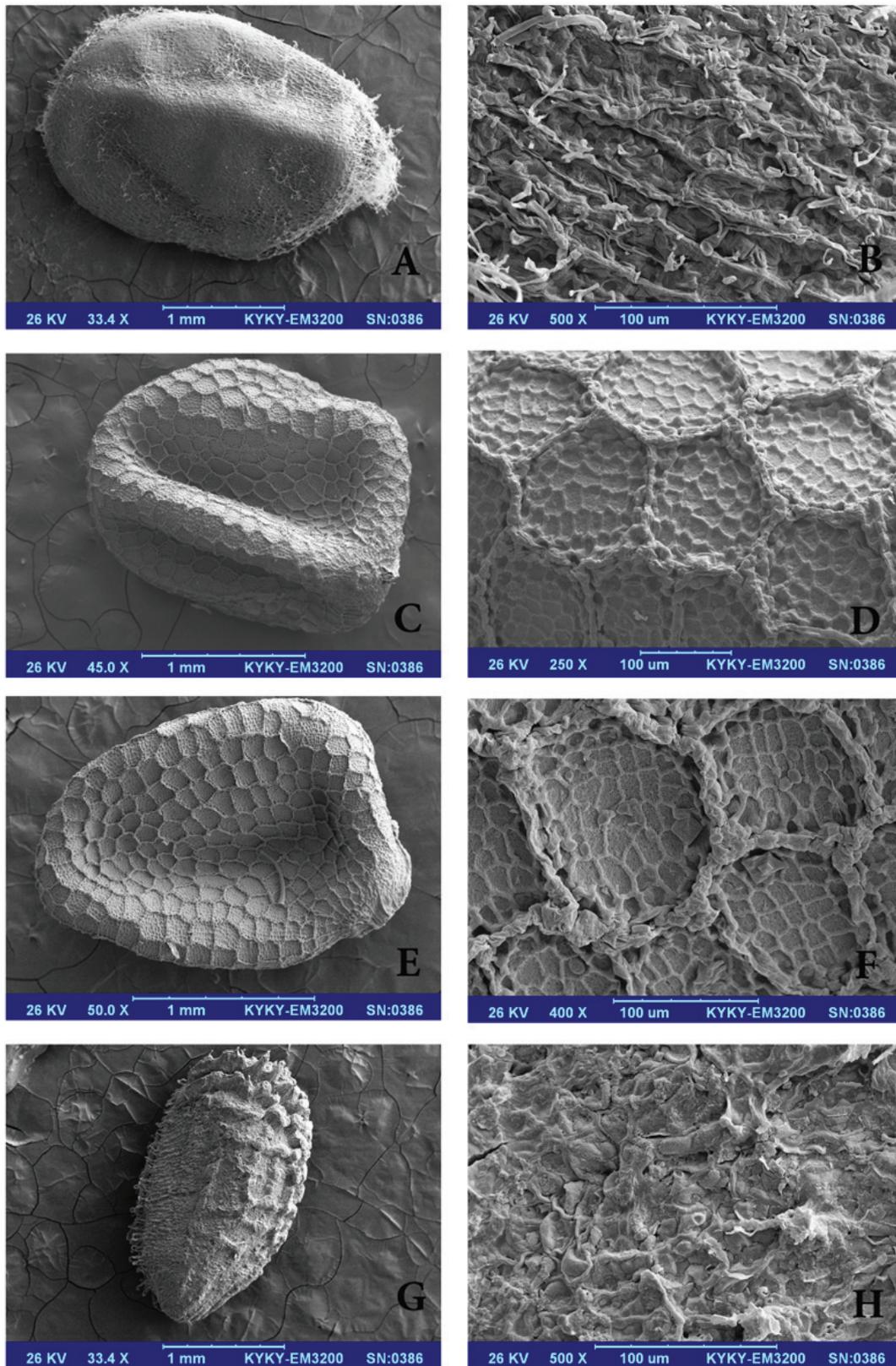


Figure 5. Scanning electron micrographs of *Geranium* seeds. Lateral views (A, C, E and G; scale bars: 1 mm) and surface ornamentations (B, D, F and H; scale bars: 100 µm) of seeds of *G. albanum*, *G. columbinum*, *G. dissectum* and *G. lucidum*, respectively.

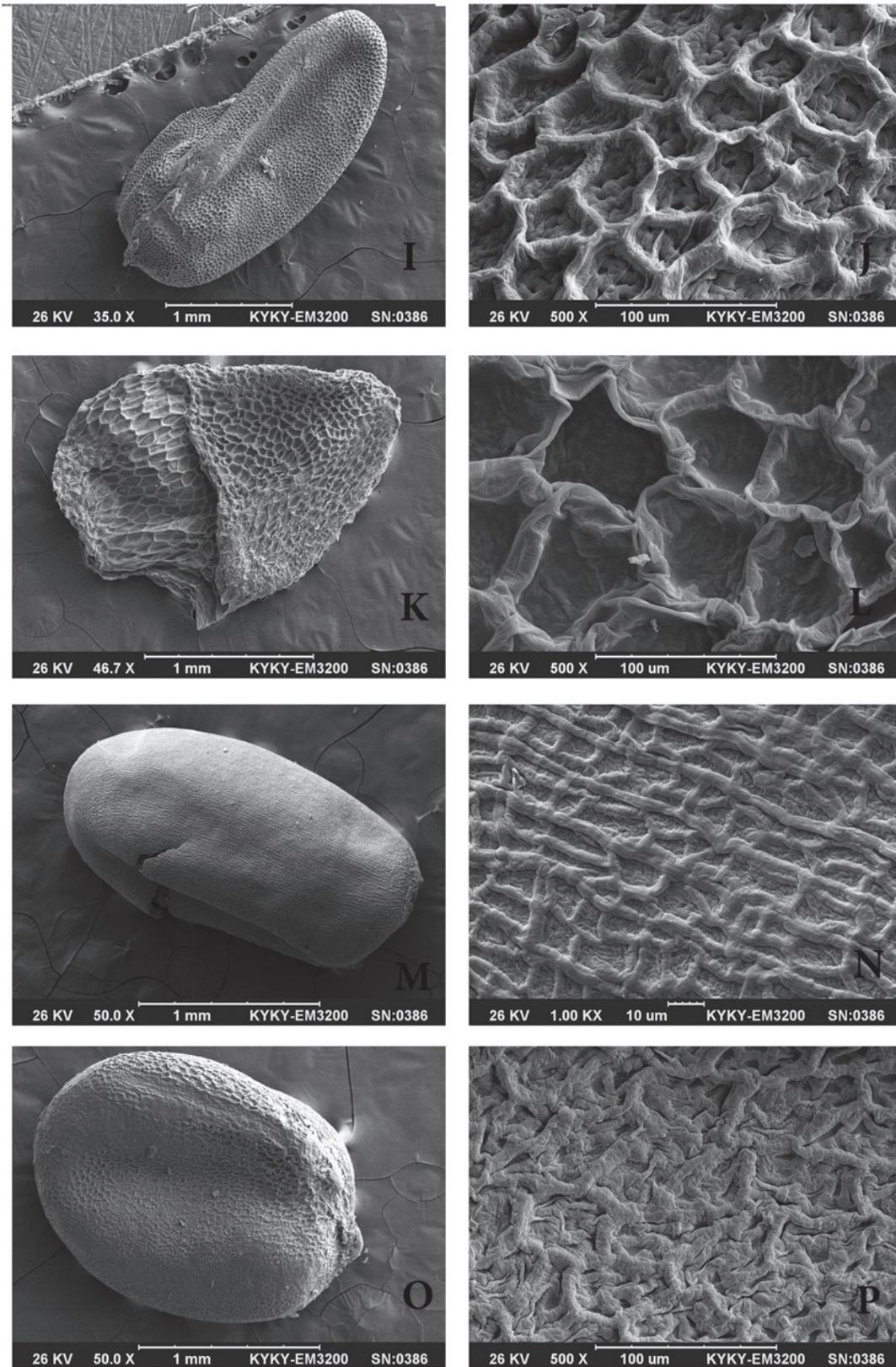


Figure 6. Scanning electron micrographs of *Geranium* seeds. Lateral views (I, K, M and O; scale bars: 1 mm) and surface ornamentations (J, L, N and P; scale bars: 100 µm) of seeds of *G. mascatens*, *G. molle*, *G. purpureum* and *G. tuberosum*, respectively.

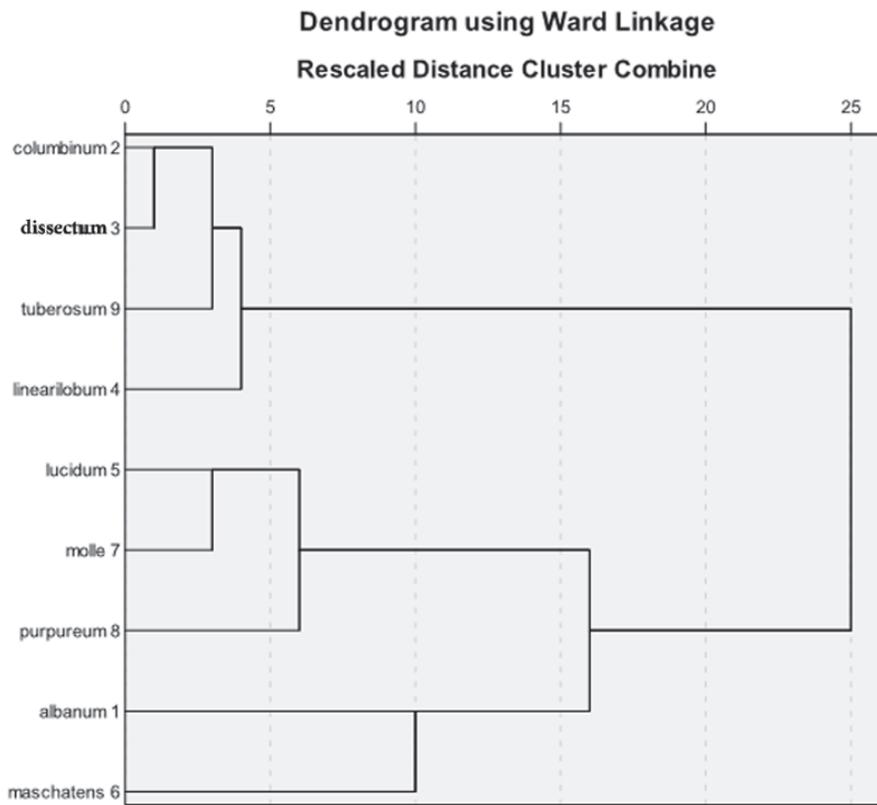


Figure 7. Dendrogram using Ward linkage for the 9 studied *Geranium* species.

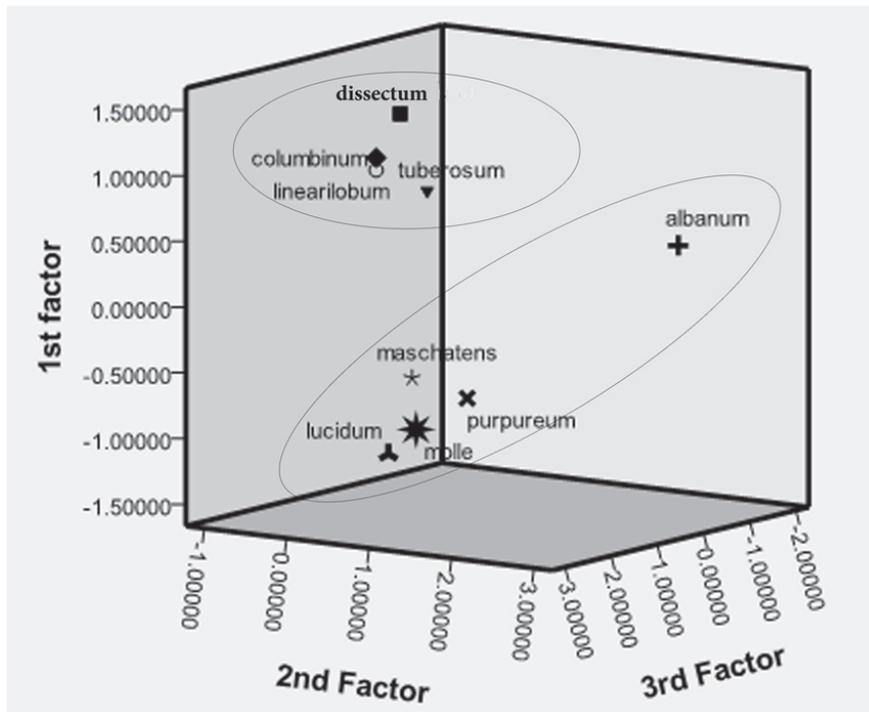


Figure 8. PCA scatter diagram of studied *Geranium* species.

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