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MACRO- AND MICROMORPHOLOGICAL STUDIES OF *CLYPEOLA* SPECIES (BRASSICACEAE) IN IRAN

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Abstract. *Clypeola* is an annual genus from Brassicaceae with four (*C. jonthlaspi, C. aspera, C. lappacea* and *C. dichotoma*) species in Iran. These are plants of different habitats and found as early spring therophytes in semiarid regions of Iran. In this study 63 populations of *Clypeola* genus have been studied by 49 macro- and micromorphological features. Results were analyzed by use of multivariate statistical methods. Cluster analysis, factor analysis and ordination methods were applied. The result showed that such characters as pedicle, trichome, stamen, petal features and sculpture of fruit surface have valuable diagnostic in separating of these species. In present study it was also found that the use of seed surface character in not effective alone for taxa delimitation except of some *C. jonthlaspi* subspecies. Among leaf features, trichome ornamentations, their position and branching pattern are effective for species separation. Fruit hairs are of diagnostics importance in species separation too.

Key words: Brassicaceae, *Clypeola*, fruit, micromorphology, seed

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Introduction

Brassicaceae comprise of 338 genera and 3709 species (Franzke *et al.* 2010). In Brassicaceae, the importance of micromorphological characters such as seed surface and hairs has been emphasized (Al-Shehbaz *et al.* 2006). Trichomes are divided into three groups based on their form, the branching pattern, types and number of cells. The trichome features are valuable in the classification of Brassicaceae on generic and specific levels. For example, Rollins & Banerjee (1976) studied the leaf trichomes of *Lesquerella* S. Watson. Ancew & Goranova (2006) studied the seed and leaf trichome morphology of eight taxa from Alysseae tribe. Mummenhoff *et al.* (1997) pointed to the characters convergence in fruit morphology during their studies on *Thlaspi* s.l. based on ITS analysis.

Based on early researches on seed surface features, characters of anticlinal and periclinal cell walls have been considered significant (Abdel Khalik *et al.* 2002; Tantawy *et al.* 2004; El Naggar *et al.* 2005; Moazzeni *et al.* 2007; Kasem *et al.* 2011). In particular, Abdel Khalik *et al.* (2002) studied morphological features of 45 taxa of Brassicaceae. They pointed on the diagnostic importance of leaf, flower, fruit, seed, embryo and trichome morphology. Bolourian & Pakravan (2011) mentioned that silicle and stamen features are valuable in separation of annual species.

Clypeola L., is an annual genus of Alysseae tribe. Distribution of this genus is limited to northern hemisphere. There are 9 species in this genus (Chaytor & Turill 1935). Rechinger (1968) mentioned 5 *Clypeola* species in Iran: *C. aspera* (Gruer) Turill, *C. lappacea* Boiss, *C. dichotoma* Boiss, *C. jonthlaspi* L. and *C. microcarpa* Morise. *C. jonthlaspi* is idely distributed in Iran and in some papers presence of varieties or subspecies of this taxon is mentioned (Chaytor & Turill 1935; Breistroffer 1936). Due to the different description of *C. microcarpa* in literatures, it is mainly considered as a subspecies or a variety of *C. jonthlaspi*. In present study *C. microcarpa* as a separate species is rejected too.

The aim of this research is to evaluate the macro- and micromorphological variation

in *Clypeola* in order to find more diagnostic features and to realize a better separation of taxa.

Material and methods

The herbarium specimens and freshly collected plants from 63 populations belonging to 4 annual taxa (*C. jonthlaspi, C. aspera, C. lappacea, C. dichotoma*) from different localities in Iran were studied (Tab. 1). Vouchers of the collected plants are deposited in Alzahra University Herbarium (ALUH). Ten individuals were taken from each locality and used for morphological studies based on species distribution patterns. Totally 49 qualitative and quantitative morphological characters, consisting of vegetative and reproductive structures were assessed (Tabs 2 & 3). Characters were selected on the base of Floras and our own field studies. The fruit, seed and leaf surface were studied by use of scanning electron microscopy (SEM). Samples were fixed on aluminum stubs using double-sided adhesive and were coated with a thin layer of gold-palladium. The SEM micrographs were taken by Philips XL30. In text El Naggar (2005) terminology is applied.

In phenetic analysis the mean of quantitative characters were used while qualitative characters were coded as binary/multistate characters. Variables were standardized (mean = 0, variance = 1) for multi-variant statistical analyses (Ng *et al.* 1981). In order to find the species relationships cluster analyses using UPGMA (un-weighted paired group, mean average) and WARD (minimum variance, spherical clusters) as well as ordination based on principal component analysis (PCA) were performed. In order to determine the most variable morphological characters among the species/populations, factor analysis based on principal components analysis (PCA) was performed. Non-variant characters were omitted before factor analysis.

Results

Macromorphological studies

Form of pedicle show variation in studied species. The pedicle is recurved and this is the diagnostic trait for *Alyssum* and *Clypeola* separation. Pedicle is like an umbrella hand in *C. jonthlaspi* (Fig. 1 E), domical in *C. aspera* (Fig. 1 F), recurved with a sharp angle in *C. lappaceae* (Fig. 1 G), and S-shaped in *C. dichotoma* (Fig. 1 H).

Fruit trichomes in studied species are columnar or pyramidal. In *C. jonthlaspi* and *C. dichotoma*, trichomes are columnar. In *C. aspera* and *C. lappacea*, trichomes are pyramidal and branching at the tip and in mature fruit. The branching pattern in different populations of *C. lappacea* is varied and in *C. aspera* it is pentameral. The trichome features in *C. jonthlaspi* showed variation and due to this it has been used to distinguish subspecies and varieties in Floras and papers. In *C. lappacea* there is a great variety in hair distribution and length.

Fruit shape is elliptic, orbicular or obovate. The style in *C. dichotoma* and *C. lappacea* is long and the tip of fruits are not emarginate (Fig. 1 I, J), though in *C. aspera* and *C. jonthlaspi* it is emarginate and style length is often equal to its width (Fig. 1 K, L). In *C. jonthlaspi, C. aspera* and *C. dichotoma* there are two distinct parts, fruit disk and wing. **The shape of cells in fruit margins** differs among three species, they are lobate in *C. jonthlaspi* (Fig. 2 A), stellate with the presence of stomata in *C. aspera* (Fig. 2 B), and represented by extensive cell with fine lobes in *C. dichotoma* (Fig. 2 C). In *C. lappacea* there is no distinction between central and marginal parts of fruit. The seed is wingless and without mucilage. Seed is elliptic, obovate, oblong and orbicular with different degree of elongation. In all studied species, different form can be seen among populations and even individuals of a population. **The calyces** are shed after ripening of fruits. In *C. aspera*, *C. jonthlaspi* and *C. dichotoma*, calyx is saccate. In *C. lappacea*, calyces are nearly straight. Sometimes the margins are purple (*C. jonthlaspi*).

Petals in studied species are oblong or triangular (*C. jonthlaspi*). In *C. aspera* and *C. lappacea*, lamina is flattened. In *C. dichotoma*, at the base of one or two petals, there is a protruding in some flower (Fig. 2 F). The

Tab. 1. Collections data for populations used in macromorphological study. * – stands for accessions used in micromorphological studies by SEM. **ALUH** – vouchers preserved in Herbarium of Alzahra University; **FARH** – in Herbarium of Kharazmi University; **FUMH** – in Herbarium of Ferdouwsi University; **HSBU** – in Herbarium of Shahid Beheshti University.

Nr	Species	Voucher Nr	Origin	Collector
1	*C. jonthlaspi*	18917-ALUH	Tehran: Boumehen	Abbasian
2		18918-ALUH	Alborz: Baghestan	Mosaferi
3		188-ALUH	Alborz: Aghesht to Baraghan *	Keshavarzi
4		18919-ALUH	Gilan: Jirandeh	Dadmehr
5		18920-ALUH	Qazvin: Abyek	Abbasian
6		18921-ALUH	Qazvin: 20 km Ange road, Shekarnab village *	Abbasian
7		18922-ALUH	Fars: Shiraz to Isfahan, 15 km to Saadat Shahr	Abbasian
8		18923-ALUH	Kermanshah: Taq-e-Bostan	Gholami
9		18924-ALUH	Tehran: Dar Abad	Abbasian, Habibi, Dadmehr
10		18925-ALUH	Tehran: Darakeh River near Vanak	Keshavarzi, Abbasian, Habibi
11		18926-ALUH	Tehran: Bumehen to Tehran road	Abbasian
12		18915-ALUH	Fars: 35 km Shiraz, 1482 m	Abbasian
13		18913-ALUH	Yazd: Tabas, Neyzar village, 1010 m	Abbasian
14		18911-ALUH	Yazd: Tabas, Eshqabad road, Ozbak Kuh	Abbasian
15		18999-ALUH	Khorasan: Boshrouyeh, Neygenan village	Abbasian
16		18918-ALUH	Fars: 35 km to Neyriz, 1480 m	Abbasian
17		1897-ALUH	Fars: Marvdasht road, Naqsh-e-Rostam	Abbasian
18		1895-ALUH	Yazd: Tabas, Khevr village	Abbasian
19		1892-ALUH	Yazd: Tabas, Eshqabad road, Kalshane village,1092 m *	Abbasian
20		1903-ALUH	Kurdistan: Sanandaj road, 15 km Kamyaran	Abbasian
21		1904-ALUH	Kermanshah: Sanandaj road	Abbasian
22		1905-ALUH	Kermanshah: 60 km Kermanshah, Bid Sorkh ghaut	Abbasian
23		24711-FUMA	Khorasan: 70 km to Mashhad, Kalat road, 1600 m *	Faqhihi nia & Zanguee
24		16372-FUMA	Khorasan: W. Dargaz-Gadganlou, 1200 m	Joharchi & Zanguee
25		25371-FUMA	Khorasan: Kashmar, SW Kuh Sorkh	Faghihi nia & Zanguee
26	*C. aspera*	18916-ALUH	Fars: 35 km to Shiraz, 1482 m	Abbasian
27		18914-ALUH	Yazd: Tabas, Neyzar village, 1010 m	Abbasian
28		18912-ALUH	Yazd: Eshqabad road, Ozbak Kuh	Abbasian
29		18910-ALUH	Khorasan: Boshrouyeh, Neygenan village *	Abbasian
30		1898-ALUH	Kerman: Kerman, 1770 m	Abbasian
31		1896-ALUH	Fars: 35 km to Neyriz, 1480 m	Abbasian
32		1893-ALUH	Yazd: Tabas, Abid village	Abbasian
33		1894-ALUH	Yazd: 40 km Deyhuk, 1361m	Abbasian

Tab. 1. Continued.

34	*C. aspera*	5044-ALUH	Fars: 35 km Marvdasht *	Rastipishe
35		1891-ALUH	Yazd: Tabas, Eshqabad road, Kalshane village, 1092 m	Abbasian
36		36394-FUMA	Yazd: SE Tabas, NE Deyhuk, 1700 m	Zanguee & Rafei
37		18917-ALUH	Qazvin: Abyek	Abbasian
38		Anonymus-HSBU	Kerman: Kerman	Sonboli
39	*C. dichotoma*	1907-ALUH	E Azerbayjan: 48 km NW Marand, salt hills, 1036 m	Akhani & Samadi
40		16313-FUMH	Khorasan: between Quchan and Dargaz, Allah Akbar ghaut, 1650 m	Joharchi & Zanguee
41		24743-FUMH	Khorasan: Bshrouyeh road, 1400 m	Rafei & Zanguee
42		26738-FUMH	Khorasan: SE Birjand, 1650 m	Rafei & Zanguee
43		Anonymus-FUMH	Khorasan: Birjand to Tabas, 1150 m	Anonymus
44		10076-FUMH	Khorasan: Birjand to Sarchah road, 1400 m	Anonymus
45		16609-FUMH	Khorasan: SW Bojnurd, 1050 m	Joharchi & Zanguee
46		17287-FUMH	Khorasan: E Birjand, Gzyk mountains, 1400-1500 m	Joharchi & Zanguee
47		13743-FUMH	Khorasan: Ferdows, Boshrouye	Ayatollahi & Joharchi
48		21809-FUMH	Khorasan: Birjand, Hamand, Give road, 1400 m.	Faghihinia & Zanguee
49		15109-FUMH	Khorasan: Birjand, 8 km after Hamand, 1300-1350 m *	Rashed & Zanguee
50	*C. lappacea*	Anonymus-HSBU	n.a.	Anonymus
51		36-HSBU	Kermanshah: Songhor, Asadabad, 1650 m	Zehzad
52		87410-HSBU	Isfahan: between Shahreza and Vanak, 2300 m	Khosravi
53		87532-HSBU	Chahar Mahal Va Bakhtiari: between Kharadgee and Gandoman, Pare Das mountain, 2150-2300 m *	Zehzad
54		Anonymus-FARH	Kordestan: Sanandaj *	Anonymus
55		18814-FARH	Iran: Anaran mountain, Ghooch Ali *	Anonymus
56		18905-FARH	Isfahan: Faridan *	Bagheri
57		18904-FARH	Kermanshah	Hassan pour
58		18906-FARH	W. Azerbaijan: Takab *	Anonymus
59		11089-FARH	Hamadan: Tuyserkan	Haj Mohamad Sameeii
60		1901-ALUH	Lorestan: Malayer-Borujerd road, 60 km to Borujerd	Abbasian
61		1902-ALUH	Kermanshah: Bid Sorkh gauth	Abbasian
62		74413-HSBU	E Azarbaijan: between Tabriz and Marand	Zehzad
63		8624402-HSBU	Chahar Mahal Va Bakhtiari: between Gandoman and Ardal, south hills of pond Shaloo, 2350-2450 m	Zehzad

Tab. 2. Qualitative macro- and micromorphological features used in this study.

Characters	State of character and coding
Vegetative form	ascending (0), erect and un-branched (1), branched at base (2), ascending with few branches (3), ascending or erect and bifurcate branches (4)
Fruit shape	ovate (0), elliptic (1), round (2), elliptic-ovate (3)
Seed shape	oblong (0), elliptic (1), elliptic-ovate (2)
Petal shape	connate (0), oblong (1), oblong-connate (2)
Hair in fruit disc	absent (0), present (1)
Hair in fruit margin	absent (0), present (1)
Pedicle shape	curved (0), recurved sharply down (1), S-shaped (2), umbrella handle like (3)
Leaf shape	elliptic and flat (1), oblanceolate-linear (2), oblanceolate and flat-elliptic (3)
Hair shape	cylindrical (0), conical (1)
Anthers shape	non-elliptic (0), elliptic (1)
Petal base protruding	present (0), absent (1)
Protruding at the end of filament and its wing	present (0), absent (1)
Fruit margin	smooth (0), crenate (1), serrate (2)
Different tissues in fruit margin	absent (0), present (1)
Fruit hair surface	without furrows (0), horizontally furrowed(1)
Fruit hair surface	tuberculate (1), hispid (2)
Hair apex	rounded (1), swollen (2), branched (3)
Trichome surface ornamentation	non-verucate (0), verucate (1)
Trichome shape	columnar (1), pyramidal (2), infundibular (3)
Trichome tapering	gradually (1), suddenly (2)
Trichome	without net-like structure (0), with net-like structure (1)
Fruit ornamentation	smooth with button shaped particles (0), with cone shaped protruding (1), tuberculate (2), papillat with irregular pattern (3), small tubercules (4), amorphous and branched (5)
Fruit surface	with deep holes(0), net-like (1), lineolate (2)
Seed surface reticulation	regular (1), irregular (2)
Seed surface	domate (0), net like (1), stripped (2), lineolate (3)

stamens are winged and wings are terminated to a lobed or acute tip. In some individuals, the stamens with two wings can be seen. Anthers are medifix in *C. aspera*, *C. jonthlaspi* and *C. dichotoma*. In *C. lappacea*, at the margin of tip of filament and wings, there are sinuate protrudings (Fig. 2 G). Length of filament and wing of stamen are valuable traits in species delimitation. Some populations of *C. jonthlaspi* showed different combination of flowers (with unusual structures or with both high density of hair and unusual structures) (Fig. 1 C).

Micromorphological studies

Fruit and seed surface were studied. Fruit and seed sculpture pattern and fruit hairs showed a great variation in *C. jonthlaspi* and *C. lappacea*.

The surface of fruit in *C. jonthlaspi* haired on both part of fruits, covered with papilla smaller than hairs, and a net-like sculptured, but in fruits with marginal hairs, surface of fruit have lineolate sculpturing pattern. In *C. aspera*, surface of fruit covered with cone-shaped protrudings which are branched at tip. The shape of these protrudings and fruit sculpturing

Tab. 3. Quantitative macro- and micromorphological features used in this study.

Characters	Characters
Leaf length	Seed length
Leaf width	Seed width
Petal length	Fruit length
Petal width	Fruit width
Sepal length	Length of style
Sepal width	Average length of fruit hair
Length of pedicle	Length of Fruit to seed ratio
Length of inflorescence	Length of longest filament/length of same anther
Length of stamen	Fruit/fruit hair length ratio
Width of fruit/length of hair	Sepal/petal length ratio
Length of fruit margin	Fruit to style length ratio
Length of pedicle/length of fruit	Fruit to seed length ratio

vary among studied populations (Fig. 3). The surface of *C. dichotoma* covered with papilate hairs which are smaller than fruit hairs. In most studied populations of *C. lappacea*, the surface of fruit is covered with branched protrudings, the form of them are different among populations. In Kurdistan population, surface of fruit is stripped and have button-shaped protrudings that scattered irregularly, while vacuolar pattern in Faridan and Paredas populations were observed (Fig. 3). In Azerbaijan population, the fruit is covered with net-like sculptures. In other populations, surface of fruit have stripe or smooth sculpture pattern as in Ilam, Kermanshah, Gandoman, and Faridan (Fig. 3).

Trichome surface has button-shaped ornamentation in *C. jonthaspi* and *C. dichtoma* but barbed-shaped in *C. aspera* (Fig. 4) and *C. lappacea*. There was an infudibular-shaped hair base in Faridan accession or the gradually narrowing at the tip of hairs (Fig. 4).

Seed surface sculpturing is mostly reticulate but three other types were observed too: domate (*C. dichotoma*), lineolate (in *C. jonthlaspi* with marginal hair at fruit), or net-like pattern (in *C. lappacea*) from Azerbaijan (Fig. 4). Other populations of *C. lappacea, C. aspera* and *C. jonthlaspi* have reticulate sculpture (Fig. 4).

A variation in **hair density** was observed while studying leaf dorsal epidermis. The hairs have button-shaped sculptures (Fig. 5 A-C). In *C. dichotoma* sculptures are not prominent and hairs are more slender (Fig. 5 D). In some studied populations of *C. lappacea*, tip of hairs is curved (Fig. 5 E).

It was evident that the lower and upper **epidermis of petal** is different. In all examined samples, the sculpture of lower epidermis is stripy and these lines are parallel and margin of cells are wavy (Fig. 5 F), but in upper epidermis of four studied species there are differences (Fig. 5 G-J).

Interspecific variation based on macro-morphological characters

The studied species showed significant differences in selected set of characters. The Ward's phenogram based on morphological features showed that there are two main clusters (Fig. 6).

The first cluster consists of *C. jonthlaspi, C. aspera* and *C. dichotoma*. In second cluster *C. lappacea* is grouped. 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 70% of total observed variation. In the first factor, with more than 50% of the total variation, characters as petal length and width, sepal length and width, filament length and width of all stamens wing, length of pedicle, length of anther, length of style, presence

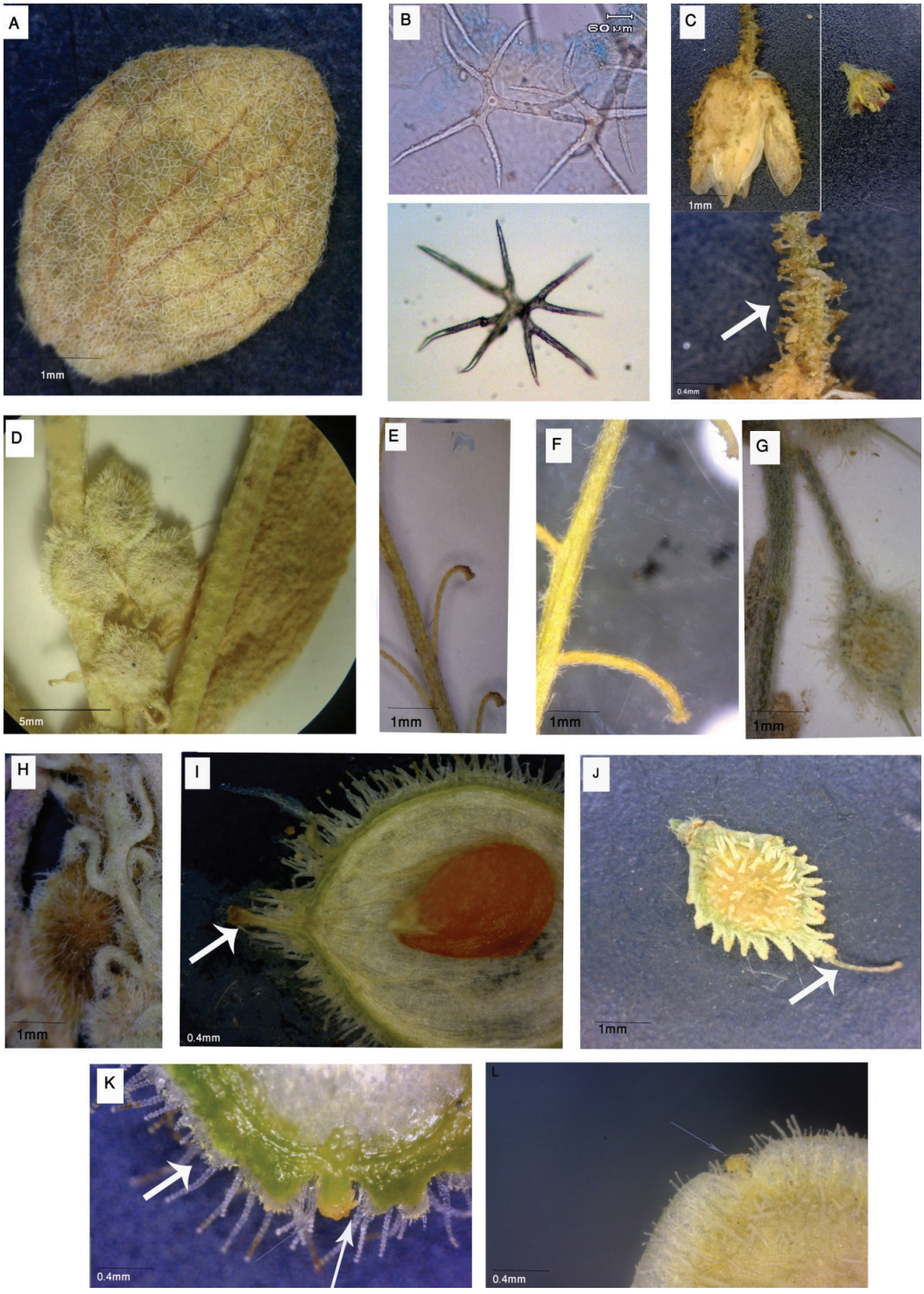


Fig. 1. A – leaf of *Clypeola dichotoma*; **B** – trichome with four main branches at down and three at upper part; **C** – abnormal (left) and normal (right) flowers of *C. jonthlaspi*; **D** – inflorescence of *C. dichotoma*, **E** – pedicle of *C. jonthlaspi*; **F** – pedicle of *C. aspera*; **G** – pedicle of *C. lappacea*; **H** – pedicle of *C. dichotoma*; **I** – style of *C. dichotoma*; **J** – style in *C. lappacea*; **K** – style and crenate shape of fruit margin in *C. aspera*; **L** – style in *C. jonthlaspi*.

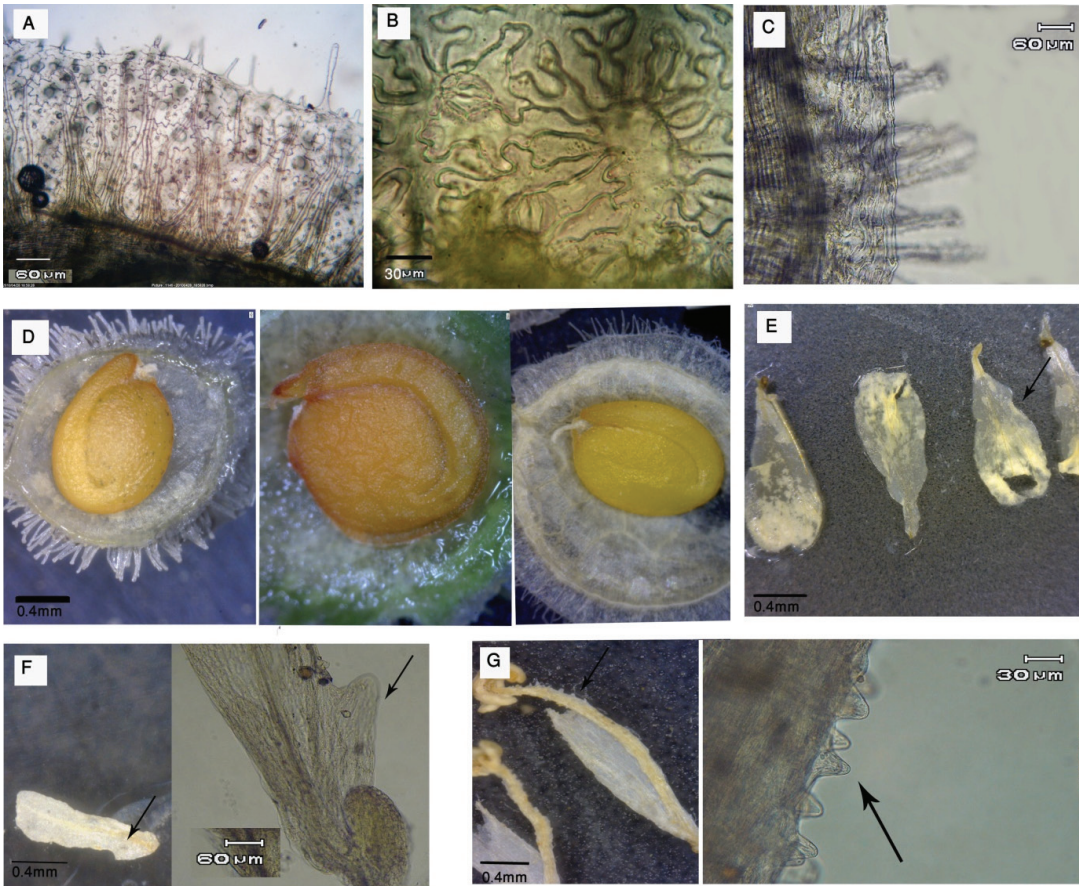


Fig. 2. Fruit margin in *Clypeola jonthlaspi* (**A**), *C. aspera* (**B**) and *C. dichotoma* (**C**); **D** – mature fruits; **E** – emargination in lower part of petal of *C. lappacea*; **F** – protruding in basal petal of *C. dichotoma*; **G** – protruding in tip of filament and wing of stamen of *C. lappacea*.

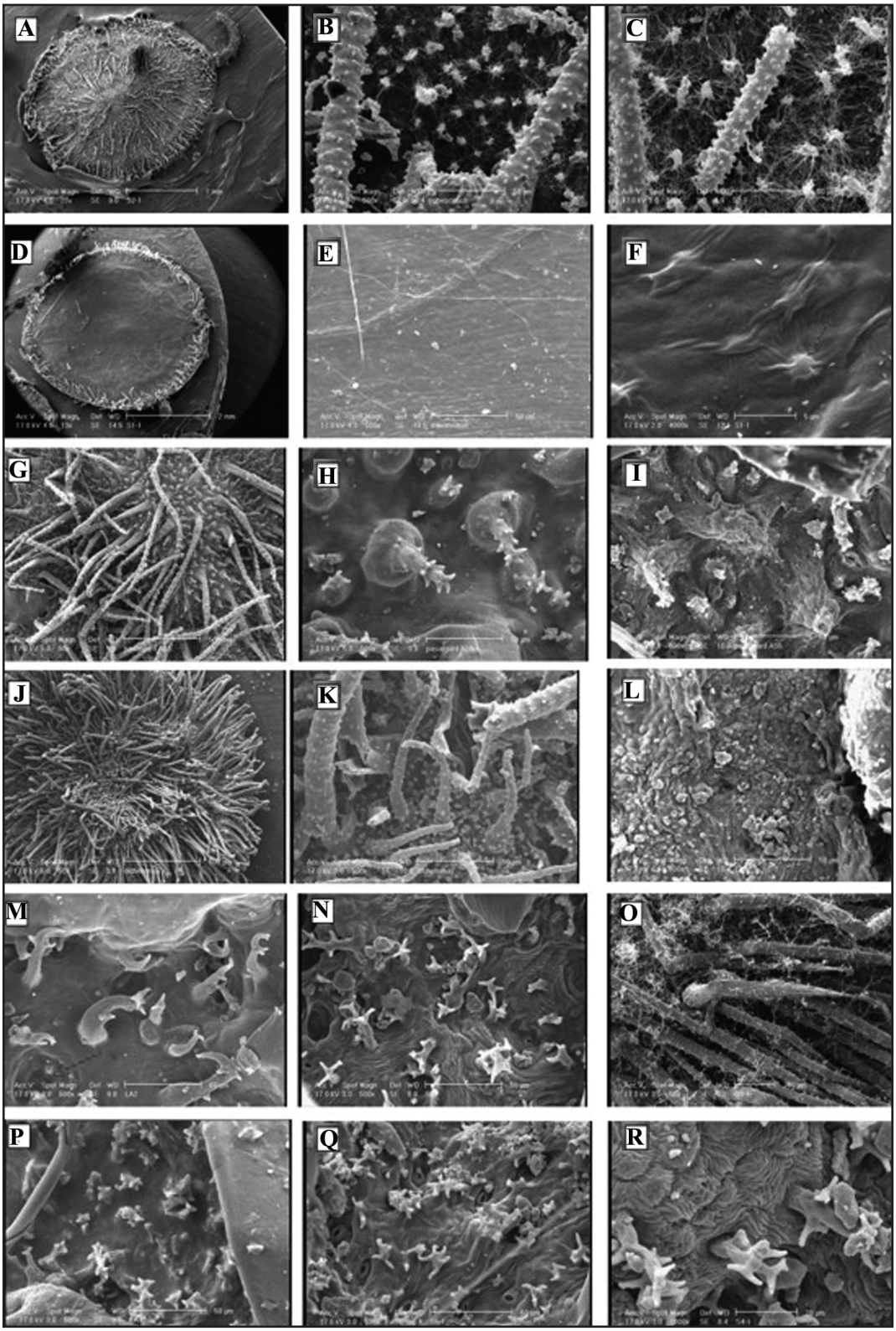
of different tissue at fruit margin, presence of protruding in filament and wing margin of stamens, shape of leaf and anther had highest correlation (>0.6). In the second factor, with about 11.53% of the total variation, characters as the height of plant, width of leaf, length of inflorescence, presence of protruding in base of petal and number of leaf veins had highest correlation(>0.6). In the third factor with about 9.44% of the total variation, characters as length of fruit trichomes, ratio of fruit length to seed length, ratio of length of filament to anther length and shape of fruit margin had highest correlation(>0.6). Therefore, these are the most variable morphological characters among the studied characters of studied *Clypeola* species.

Interspecific variation based on micromorphological characters of seed and fruit

In order to group the species having micromorphological similarities, cluster analysis using Ward's method was performed (Fig. 7). It has revealed two main clusters (Nrs 1 & 2).

In cluster Nr. 1, *C. jonthlaspi, C. dichotoma* and one population from *C. lappacea* are grouped. There are two subsets in this cluster, one of which contains populations from Kalshane and Karaj belonging to *C. jonthlaspi*.

Fig. 3. Seed and fruit surface in *Clypeola jonthlaspi* (**A-F**), *C. aspera* (**G-I**), *C. dichotoma* (**J-K**), and *C. lappacea* from ▸ different populations (**L-R**). **L** – Kordestan; **M** – Faridan; **N** – Paredas; **O** – Azerbaijan; **P** – Isfahan; **Q-R** – Kermanshah.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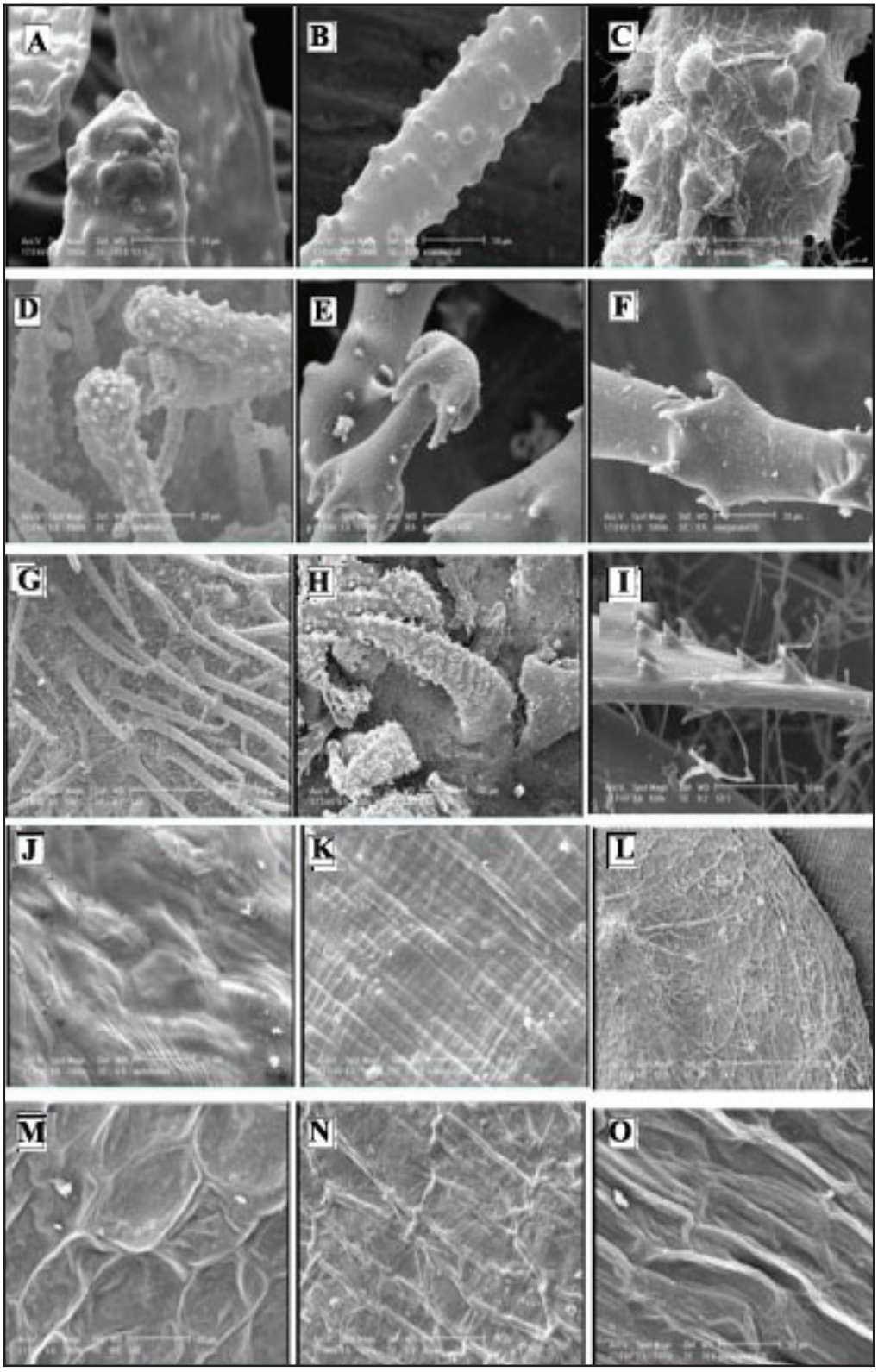
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In these populations, trichomes of fruit can be seen only at margin of fruit. In **A** cluster populations of Karaj, Qazvin and Mashhad-Kalat of *C. jonthlaspi* are grouped. These taxa have fruit hairs in both parts. Presumably, the reason for grouping population of Azerbaijan from *C. lappacea*, in this subset, is the presence of net like sculpture on the surface of fruit (Fig. 3), which can be seen in populations of *C. jonthlaspi* (Fig. 3 A). Cluster Nr. 2 contains

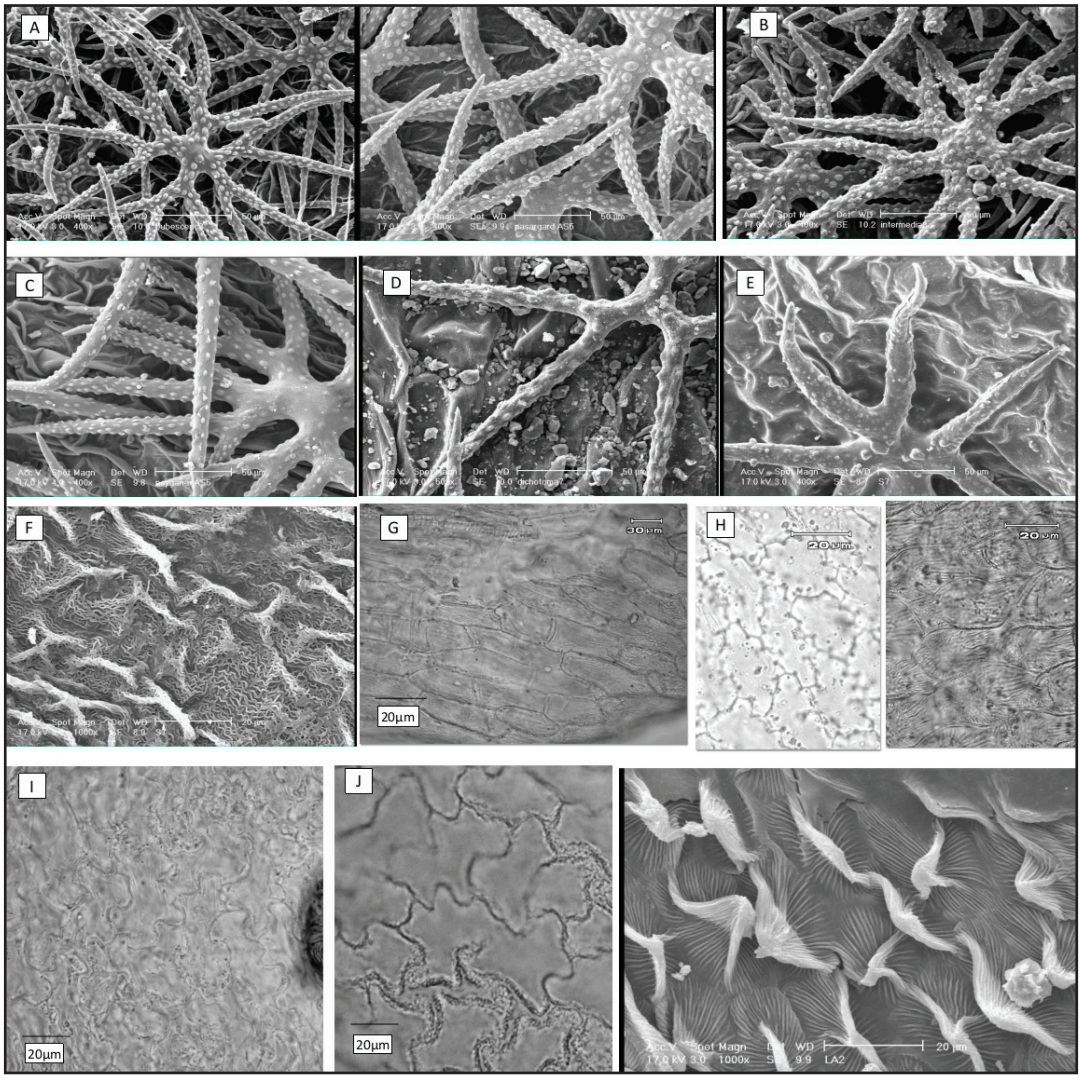

Fig. 5. Leaf and petal surface. **A-E** – leaf hairs; **A** – hair with protruding that are larger in center; **B** – hair with equal protruding in all parts; **C** – hairs without protruding in center; **D** – hair of *C. dichotoma*; **E** – hair with curvature in tip in *C. lappacea*; **F** – dorsal petal epidermis in *C. lappacea*; **G-J** – petal ventral epidermis in *C. jonthlaspi* (**G**), *C. aspera* (**H**), *C. dichotoma* (**I**), and *C. lappacea* (**J**).

◂ **Fig. 4.** Fruit and seed surface. **A-I** – fruit surface. **A-C** – rounded trichome tip and button shaped sculptures in *Clypeola jonthlaspi*; **D** – swollen trichome tip in *C. dichotoma*; **E-F** – branched tip and barbed sculptures in *C. aspera*; **G** – infundibular-shaped trichomes in *C. lappacea*; **H** – narrowed gradually trichomes in *C. lappacea*; **I** – sharping tip in *C. lappacea*. **J-O** – seed surface sculptures. **J** – domate in *C. dichotoma*; **K** – lineolate in *C. jonthlaspi*; **L** – net-like in *C. lappacea*; **M** – reticulate in *C. aspera*; **N** – reticulate in *C. lappacea*; **O** – reticulate in *C. jonthlaspi*.

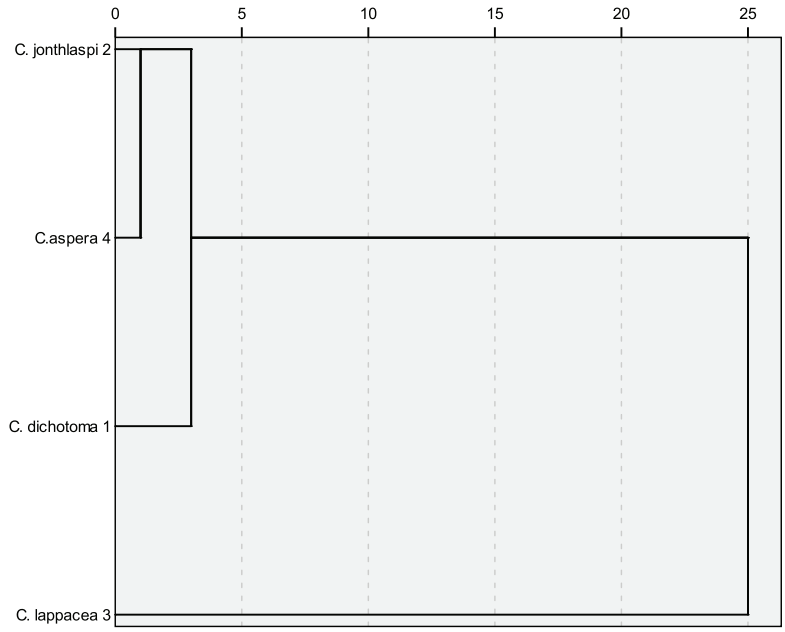

Fig. 6. Phenogram (Ward's method) based on macromorphological characters in four annual *Clypeola* species.

populations of *C. lappacea* and *C. aspera*. In **F** cluster populations of *C. aspera* are clearly separated. The arrangement of *C. lappacea* and *C. jonthlaspi* populations in dendrogram are modified for macro- and micromorphology with pollen morphology results. In order to determine the most variable characters among the studied species, a factor analysis based on PCA was performed, revealing that the first three factors comprise about 61% of total variation. In the first factor with about 31% of the total variation showed the highest correlation (>0,6). Second and third factor with about 16% and 15% of the total variation respectively showed the highest correlation.

Discussion

By considering of different populations of *Clypeola* species in Iran in present study it was evident that, some morphological characters as pedicle shape, shape of fruit hair, shape of fruit margin, characters of stamen, shape of style and petals are efficient in species separation. A number of authors (Abdel Khalik 2002; Tantawy *et al.* 2004; El Naggar 2005; Moazzeni *et al.* 2007; Kasem *et al.* 2011) demonstrated that seed surface features are valuable in separating species regarding anticlinal and periclinal walls. In this study we demonstrated that the use of seed surface character is not efficient when used alone, but it was also shown that some subspecies of *C. jonthlaspi* could be separated by these features. The diagnostic importance of fruit characters in present study is in concordant with Kaya *et al.* (2011).

Some micromorphological leaf features as hair position, branch number and diameter are effective in species separation. Rollins & Banejee (1976) mentioned the importance of same features while studying *Lesquerella* species. It is believed that in dry condition, hairs are denser than humid condition because of adaptive behavior of plants. Results of these study is somewhat in congruent with this idea, as in *C. aspera* and *C. jonthlaspi* and *C. lappacea* (Faridan) from drier location than Ilam and Bakhteeyari belonging to *C. lappacea* have a higher hair density. *C. dichotoma* (Birjand population) which grow in dry location, the hair density is low. Trichomes with curvature

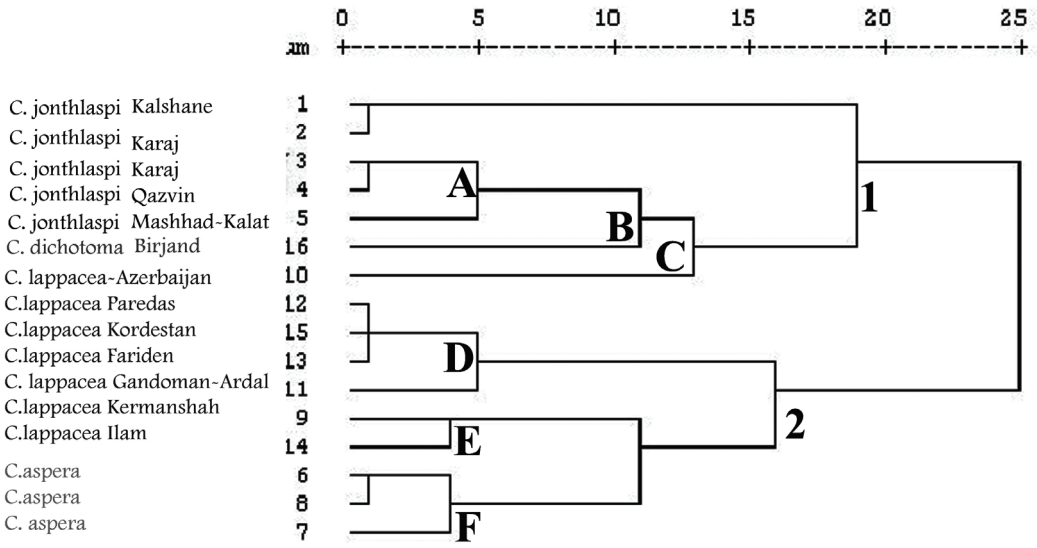

Fig. 7. Phenogram (Ward's method) based on seed and fruit micromorphological characters in four annual *Clypeola* species.

at their tips were observed in populations of Gandoman, Ardal and Ilam of *C. lappacea*.

Fruit micromorphological features especially fruit hairs have diagnostic role in species delimitation but leaf and seed characters are used as complementary. Due to the vast variation which was observed in *C. jonthlaspi* and *C. lappacea* populations, the result of micromorphological studies can be helpful to separate these species as it was evident in palynological study of same genus (Keshavarzi *et al.* 2012).

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