

Phenetic analysis of the genera medicagoid *Trigonella*, *Medicago* and *Melilotus* (Fabaceae) on seed coat in Iran

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Abstract. Khandani S, Assadi M, Nejadsatari T, Mehregan I. 2016. Phenetic analysis of the genera medicagoid *Trigonella*, *Medicago* and *Melilotus* (Fabaceae) on seed coat in Iran. *Biodiversitas* 17: 162-171. Seed morphological characters of 11 species belonging to the Medicagoid *Trigonella* group from Iran were studied and compared with six species of *Trigonella* L. (non medicagoid), one species *Medicago* L. and *Melilotus* Mill. (Fabaceae), including general shape, size, color, surface shape and testa ornamentation patterns with Scanning Electron Microscope and Stereomicroscope. Phenetic relationships among the species were studied via important seed morphological data by PCA analysis and UPMGA method. Cluster analysis of seed morphology results showed that some of medicagoid *Trigonella* species clustered with non medicagoid species but majority of them joined together which is in agreement of some references. In conclusion, seed morphological characters show that most Iranian medicagoid *Trigonella* species are well separated from the other *Trigonella* species. Also, some of these characters are important in systematic discriminations of the taxa.

Keywords: Fabaceae, medicagoid, phenetic analysis, seed morphology, *Trigonella*

INTRODUCTION

Fabaceae is the third largest family of flowering plants with about 650 genera and 1000 species. Many of the species are important as the source of food, wood, raw material, fixation of atmospheric nitrogen (N₂) and etc. (Mirzaei et al. 2015b). *Trigonella* L. is a large genus with 135 species from the tribe *Trifolieae* family Fabaceae, and is distributed in dry regions with a Mediterranean climate, in West Asia, Europe, North and South Africa, North America and South Australia (Ceter et al. 2012).

The tribe *Trifolieae* consists of six genera, *Medicago* L., *Melilotus* Mill., *Ononis* L., *Parachetus* Buch-Ham., *Trifolium* L. and *Trigonella* L. The tribe was studied by Berchtold and Presl (1820) and characterized by having trifoliolate leaves and stipules adnate to the petioles (Ranjbar and Hajmoradi 2012). Schulz (1901) put four genera *Melilotus*, *Trigonella*, *Factorouskya* and *Medicago* in the tribe *Trigonelleae* having seed with micropyle infra funiculum (Tia 2004). Small (1987) recognized *Melilotus*, *Trigonella* and *Medicago* a subtribe *Trigonellinae*. Members of this subtribe are united by the consistent presence of pinnately trifoliolate leaves and high bootstrap support (99%) in analyses of *matK* data (Steel et al. 2003).

In Flora Iranica (Rechinger 1984) the *Trigonella* genus is represented by 63 annual and perennial species in 12 sections. This taxon is known exhaling odor and is considerably useful for food and medicine (Ranjbar and Hajmoradi 2012). *Trigonella foenum-graecum* L. is known to be used beneficial health effects. (Srichamroen et al. 2011). Members of the Tribe *Trifolieae* bear trifoliolate leaves and small seeds with developed radicle (Tia 2004).

Trigonella species are hairy or glabrous annual herbs with pinnately trifoliolate leaves.

Leaflets are usually toothed, leafy stipules adnate to the petiole. Flowers are axillary, solitary or mainly in heads, spikes or short racemes, calyx 5-toothed, campanulate or sometimes tubular, regular or 2-lipped, petals are yellow or white, violet or blue. Fruit exerted from the calyx, straight or curved, linear to oblong or ovate or semilunar, with or without a beak. Seeds are tubercled or wrinkled rarely smooth (Huber-Morath 1970).

According to Steel et al. (2003), *Trigonella* and *Medicago* are well-supported clades and are sister to one another and according to Bena et al. (2001), they have proper relationship. Gazara et al. (2001) showed that the genera *Trifolium*, *Melilotus*, *Trigonella* and *Medicago* are closely related to each other, but according to Bena et al. (2001), generic delimitation is problematic. Hyne (1966) reported intermediate species between *Trigonella* and *Melilotus* (Gazara et al. 2001). Some taxonomical studies for delimitation of *Trigonella* and *Medicago* such as floral characters, asymmetric leaves, phenolic variation, stigma morphology, pollen-ovule pattern and seed characters for discriminating among and between them were not successful (Bena 2001), but Small (1986) based on some floral adaptations transferred 23 *Trigonella* species to *Medicago* and named them medicagoid group (Gazara et al. 2001). These 23 species have taxonomic problem in the genus *Trigonella* and have been considered as belonging to the genus *Trigonella*. Baum (1968) introduced them as medicagoid species because of floral and seed structure similarities between them and *Medicago* species, but based on strong similarities between *Trigonella* and these species

in the fruit appearance maintained them in the genus *Trigonella* (Bena 2001).

A molecular phylogeny supported the transfer of the medicagoid species from the genus *Trigonella* to *Medicago* (Ranjbar and Hajmoradi 2012). Analyses of nrDNA ITS and ETS data supports inclusion of the medicagoid *Trigonella* species in the genus *Medicago* (Steel 2003). Some authors have used the morphology, micromorphology and seed coat anatomy to identify *Trifolieae* species but they have seldom been used in the taxonomy of the group.

Lersten (1982) noted seed shapes, testa surface, testa color patterns, seed size, hilum and lens color to separate *Trifolieae* from *Vicieae*. Small and Brookers (1990) studied delimitation of *Medicago* from its close relatives in tribe *Trifolieae* subtribe *Trigonellinae* by Scanning Electron Microscope of seed surface in the genera *Medicago*, *Trigonella* and *Melilotus*. Tia (2004), studied some seed characters in thirty eight species distributed in tribe *Trifolieae*, which showed the genera *Trigonella* and *Melilotus* have variable characters and *Medicago* have characteristic seeds. Ceter et al. (2012) studied seed morphology of 37 taxa of *Trigonella* from various regions in Turkey with Scanning Electron Microscope and light microscope such as seed color, shape, size, weight and surface ornamentation pattern.

Salimpour et al. (2013) showed cluster analysis based on morphological characters of *Medicago* and *Trigonella* (*Bucerates*) species. In this study we use some seed characters to identify relationship between medicagoid species of *Trigonella* and the genera *Medicago* and *Melilotus*. Turki et al. (2013) studied seed morphology of 19 *Trigonella* L. Species such as *T. coerulescens* (M. Bienb.) Halacsy, *T. calliceras* Fisch. ex M. Bieb. and *T. monspeliaca* L. with light and Scanning Electron Microscope. They compared them in seed characters such as shape, hilum shape, position, seed coat pattern and evaluated their taxonomic significances.

The aim of this study is to evaluate seed morphological characters in the taxonomy of the genus *Trigonella*, especially *Trigonella* medicagoid and non medicagoid species.

MATERIALS AND METHODS

The materials collected from various regions of Iran during May and June 2014, and deposited specimens in TARI and IAUH Herbaria of Iran (Table 1). The collected samples were identified at the Department of Biology, Science and Research Branch, Islamic Azad University in Tehran and at the Research Institute of Forest and Rangelands by using various floras of Iran and adjacent countries. For Scanning Electron Microscopy studies, dry seeds were examined by Stereomicroscope and were photographed and then they were mounted directly on stubs by using adhesive tape and coated with gold by E5200 AUTO sputter coater for 15 minutes.

Testa ultrastructure surface ornamentations were assessed and photographed by Cam Scan MV2300 electron

microscope. For each species we used nearly 10-20 numbers from each sample. Seeds were studied by Stereomicroscope DSC-H50 to assess morphological features and few of them were examined by Scanning Electron Microscope (SEM). Testa patterns terminology was based on Lersten (1982), Small and Brookers (1990), Pinar et al. (2009), Gunes et al. (2011). Ceter et al. (2012), Gunes F. (2013), Teixeira et al. (2013), Ozbek et al. (2014) and Mirzaei et al. (2015).

A total of 8 quantitative/qualitative characters related to seed morphology were studied in 21 taxa of *Trigonella* (6 species), medicagoid (11 species), *Medicago* (1 species) and *Melilotus* (1 species) (Table 2). For statistical analysis the qualitative characters encoded according to the multi-state method and related means were considered for quantitative characters. Phenetic analysis was carried out using SPSS Ver. 22 (Chicago, USA) and UPGMA method. Means of the characters were obtained by bootstrap analysis. Bootstrap result was based on 243-997 samples. Second analysis was done with principal component analysis (PCA) using SPSS software (Figure 6).

RESULTS AND DISCUSSION

Results

Microscopy observations showed that the studied medicagoid *Trigonella* taxa have variation in qualitative and quantitative characters (Table 3).

Seed size: seed sizes are very variable (Table 3). In medicagoid *Trigonella* group the largest seed belongs to *Trigonella monantha* (average 2.35 mm in length and 0.79 mm in width) and the smallest seeds observed in *Trigonella brachycarpa* (average 1.1 mm in length and 0.57 mm in width).

Seed shape and color: Seed shapes in medicagoid *Trigonella* species are oblong, ovoid, oblong-elliptic and seed color green and brown (Figures 1 and 2).

Testa texture: Testa textures in medicagoid group are two types creased and smooth (Figures 3 and 4).

Seed coat surface: By Scanning Electron Microscopy, several types of micro-ornamentation were observed on surface structures. The taxa have several ornamentation types that are reticulate, several types of verrucate such as foveolate-verrucate, aculate-verrucate, tuberculate-verrucate, reticulate-verrucate, rugolate-verrucate and rugolate (Table 3, Figures 3 and 4).

Table 2. Seed morphological characters and score of studied species

Morphological characters	Score
Mean seed length (mm)	0=1-2, 1=2.1-3, 2=3.1-4
Mean seed width (mm)	0=0.5-1, 1=1.1-1.5, 2=1.6-2
Seed shape	Oblong = 0, ovoid=1, oblong to another shapes=2
Seed color	Light brown=0, dark brown=1, green=2
Testa structure	Smooth=0, creased =1
Homogeneity of cell sizes	Uniform=0, irregular=1
Testa ornamentation	Verrucate=0, reticulate=1, rugulate=2
Hilum shape	Elliptic=0, circular=1

Table 1. Specimen examined of the genera medicagoid *Trigonella*, *Medicago* and *Melilotus* (Fabaceae) in Iran

Taxa	Location, collector (s) and herbarium number
<i>Trigonella</i>	
Sec. <i>Bucrates</i>	
<i>T. arcuata</i> C.A. Mey.	Iran: East Azerbaijan, 2 km from Jolfa to Siahrud, 770 m, Kasebi 14503 (IAUH)
<i>T. arcuata</i> C.A. Mey.	Iran: West Azerbaijan, Tabriz to Ahar, Khajeh Station, 1470 m, Kasebi 14502 (IAUH)
<i>T. aurantiaca</i> Boiss.	Iran: Khozestan, Hafttappéh, Chaghazanbil, 160 m, H. Foroughi 3277 (TARI)
<i>T. crassipes</i> Boiss.	Iran: Kurdistan, 54 km from Sanandaj to Kamiaran, Dolab, 1500 m Maroofi & Mansori & Sh-Naseri 5770 (TARI)
<i>T. fischeriana</i> Ser.	Iran: CharmahalBakhtiari, ShahreKord, Shalamzar, Mozaffarian 54602 (TARI)
<i>T. macroglochín</i> Durieu.	Iran: Quahak, 1900 m, Dini-Arazm 16865 (TARI)
<i>T. monantha</i> subsp. <i>monantha</i> C.A. Mey.	Iran: West Azerbaijan, Uromieh, 35 km Uromieh to Sero, 1700 m, Khandani 14514 (IAUH)
<i>T. orthoceras</i> Kar & Kir.	Iran: East Azerbaijan, Tabriz to Maraghe, 2 km from Police Station to Azarshahr, 1500m, Kasebi 14519 (IAUH)
<i>T. orthoceras</i> Kar & Kir.	Iran: West Azerbaijan, Uromieh, 35 km Uromieh to Sero, 1700 m, Khandani 14518 (IAUH)
<i>T. persica</i> Boiss.	Iran: CharmahalBakhtiari, roadfromLordegantoYasuj, Maymand, Margh-e-Chenar, 1750m Mozaffarian 54563 (TARI)
<i>T. tenuis</i> Fisch.	Iran: Ardebil to Khalkhal, Bahreman, 1800 m, Ranjbar & Hajmoradi 19601 (TARI)
<i>T. uncinata</i> Banks & Soland.	Iran: Lorestan, Road of Dorood to Pirabdollah, 1350 m, Hamzeh 71867 (TARI)
Sec. <i>Biebersteiniana</i>	
<i>T. coerulescens</i> (m. B).	Iran: Tehran, Chitgar, 8017 (IAUH)
Sec. <i>Calliceras</i>	
<i>T. calliceras</i> Fisch.	Iran: Azerbaijan, Arasbaran, Kalaibar to Hijrandust, 1350-1700m, Assadi & Maassoumi 20091 (TARI)
Sec. <i>Cylindrica</i>	
<i>T. spruneriana</i> Boiss.	Iran: Tehran, Lavizan, 8010 (IAUH)
Sec. <i>Elliptica</i>	
<i>T. elliptica</i> Boiss.	Iran: ChaharmahalBakhtiari, ShahreKord, 2400 m, Mozaffarian 54578 (TARI)
Sec. <i>Falcatulae</i>	
<i>T. uncata</i> Boiss & Noe.	Iran: Khuzestan, 7991 (IAUH).
Sec. <i>Lunatae</i>	
<i>T. brachycarpa</i> Fisch.	Iran: Azerbaijan, Arasbaran, protected area, between Asheghlow and Kalaleh, 400 m, Hamzeh & Asri 79167 (TARI)
Sec. <i>Reflexae</i>	
<i>T. monspeliaca</i> L.	Iran: Gorgan, East of Marvetappéh near Chazanghayeh, 300 m, Assadi & Maassoumi 55572 (TARI)
Sec. <i>Verae</i>	
<i>T. turkmena</i> M. Popov.	Iran: Esfahan, West Khansar, 2600m, Babakhanlo & Amin 16913 (TARI)
<i>Medicago</i>	
Sec. <i>Hymenocarpos</i>	
<i>M. radiata</i> Boss.	Iran: Kohgiluyeh Boirahmad, Mehregan & Yeganeh 13978 (IAUH)
<i>Melilotus</i>	
<i>M. officinalis</i> L.	Iran: 7571 (IAUH)

Homogeneity of cell sizes: All studied taxa have uniform homogeneity except *Trigonella arcuata* (EA) (Medicagoid) and *Trigonella calliceras* (non medicagoid) have irregular homogeneity cell sizes.

Hilum shape: All studied taxa have elliptic hilum shape except *Trigonella monantha*, *T. orthoceras* and *T. persica* (Medicagoid) and *T. coerulescens* (non medicagoid) that have circular hilum shape.

Morphological analysis: Morphological analysis of 21 taxa including six species of *Trigonella* non medicagoids, 11 species of *Trigonella* medicagoids, one species of *Medicago* and one species of *Melilotus* were analyzed based on 8 morphological seed characters. Figure 5 shows a phenogram with UPGMA method. The UPGMA phenogram showed the highest phonetic correlation ($r >$

0.60). Morphological analysis showed two main clusters, cluster A and cluster B. *Trigonella elliptica*, *Melilotus officinalis* and *Trigonella calliceras* form a separate group at a farther distance from the other species are placed in cluster A. Cluster B is divided into two groups that includes group B1 and B2, and group B2 includes group B2a and B2b.

Group B1 includes mostly medicagoid species except *Trigonella coerulescens*. In subcluster B2 *Trigonella spruneriana* and *T. turkmena* (non medicagoid) form a separate group and *T. aurantiaca*, *T. monspeliaca* and *T. crassipes* (Medicagoid) species are adjacent to *T. uncata* (non medicagoid). *Medicago radiata* is considered as an out group at a farther distance from the other species.

Table 3. Macro-and micro-morphological characters of seeds in medicagoid *Trigonella* species and some relatives from Iran

Taxa	Length (mm) Mean	Width (mm) Mean	Outline	Color	Testa texture	Homogeneity of cell sizes	Testa ornamentation	Hilum shape
<i>Trigonella</i>								
Sec. <i>Bucerates</i>								
<i>T. arcuata</i> (EA)	1.17	0.64	Oblong	Light green	Creased	Irregular	Reticulate	Elliptic
<i>T. arcuata</i> (WA)	1.84	0.78	Oblong	Dark green	Creased	Uniform	Foveolate-verrucate	Elliptic
<i>T. aurantiaca</i>	1.41	0.58	Oblong-elliptic	Light brown	Smooth	Uniform	Acuate-verrucate	Elliptic
<i>T. crassipes</i>	1.72	0.77	Oblong-elliptic	Light brown	Smooth	Uniform	Reticulate-verrucate	Elliptic
<i>T. fischeriana</i>	1.25	0.68	Oblong-elliptic	Light brown	Smooth	Uniform	Tuberculate-verrucate	Elliptic
<i>T. macroglouchin</i>	1.56	0.57	Oblong	Dark brown	Creased	Uniform	Rugolate-verrucate	Elliptic
<i>T. monantha</i> subsp. <i>monantha</i>	2.35	0.79	Oblong	Green	Creased	Uniform	Acuate-tuberculate-verrucate	Circular
<i>T. orthoceras</i> (EA)	1.28	0.62	Oblong	Green	Creased	Uniform	Reticulate	Circular
<i>T. orthoceras</i> (WA)	1.67	0.74	Oblong	Dark brown	Creased	Uniform	Acuate-tuberculate-verrucate	Circular
<i>T. persica</i>	1.95	0.69	Oblong	Dark brown	Creased	Uniform	Acuate-tuberculate-verrucate	Circular
<i>T. tenuis</i>	1.95	0.79	Oblong or oblong-elliptic	Dark brown	Creased	Uniform	Acuate-tuberculate-verrucate	Elliptic
Sec. <i>Biebersteinianae</i>								
<i>T. coerulescens</i>	1.47	0.95	Ovoid	Dark brown	Creased	Uniform	Reticulate-verrucate	Circular
Sec. <i>Callicerates</i>								
<i>T. calliceras</i>	1.77	1.82	Ovoid	Dark brown	Creased	Irregular	Reticulate-verrucate	Elliptic
Sec. <i>Cylindrica</i>								
<i>T. spruneriana</i>	2.4	0.71	Allantoid-oblong	Light brown	Creased	Uniform	Acuate-reticulate-verrucate	Elliptic
Sec. <i>Elliptica</i>								
<i>T. elliptica</i>	3.68	1.94	Elliptic-ovoid	Brown	Creased	Uniform	Reticulate-verrucate	Elliptic
Sec. <i>Falcatulae</i>								
<i>T. uncatata</i>	1.28	0.61	Oblong-elliptic	Brown	Smooth	Uniform	Reticulate-foveolate	Elliptic
Sec. <i>Lunatae</i>								
<i>T. brachycarpa</i>	1.1	0.57	Ovoid	Light brown	Smooth	Uniform	Foveolate-verrucate	Elliptic
Sec. <i>Reflexae</i>								
<i>T. monspeliaca</i>	1.12	0.67	Oblong-square	Dark brown	Creased	Uniform	Reticulate-verrucate	Elliptic
Sec. <i>Verae</i>								
<i>T. turkmena</i>	2	0.63	Allantoid-oblong	Brown	Creased	Uniform	Acuate-reticulate-verrucate	Elliptic
<i>Medicago</i>								
Sec. <i>Hymenocarpos</i>								
<i>M. radiata</i>	2	1.11	Oblong	Light brown	Smooth	Uniform	Rugulate	Elliptic
<i>Melilotus</i>								
<i>M. officinalis</i>	2.25	1.52	Ovoid	Dark brown	Smooth	Uniform	Acuate-tuberculate-verrucate	Elliptic

Note: EA: East Azerbaijan, WA: West Azerbaijan

Discussion

Studies on medicagoid *Trigonella* species including seed morphology, seed size, color, coat ornamentation, hilum shapes and homogeneity of cell sizes show relationship of medicagoid *Trigonella* species. Non medicagoid *Trigonella* species including *Medicago* and *Melilotus* species are grouped separately. Some seed morphological studies of Fabaceae taxa have been carried out from time to time (Salimpour et al. 2007; Al-Ghamdi et al. 2010; Salimpour et al. 2013; Gunes et al. 2011; Gunes 2013; Turki et al. 2013; Kahraman et al. 2014; Ozbek et al. 2014; Bianco et al. 2015; Mirzaei et al. 2015; Rodriues et al. 2015).

The results are summarized in Table 3 and Figures 1-4 using Stereomicroscope and SEM studies. These results revealed that the seed features variation may be used to discriminate medicagoid *Trigonella* species from the other

taxa. Tai (2004) has noticed that the genus *Trigonella* have variable seed characters that can be used in the subscription of their species. We have noticed that the seed size in medicagoid *Trigonella* species is variable from smallest seed in *Trigonella brachycarpa* to largest seed in *Trigonella monantha* and a wide range of variety in other taxa, while Ceter et al. (2012) observed little differences in section *Lunatae* comparing to some other *Trigonella* sections. Turki et al. (2013) showed two types of seed size.

From Table 3 and Figures 1 to 4 can be resulted that the seed color in medicagoid *Trigonella* species are two types green and brown but in the other studied taxa are only brown. Shape of the seeds in studied species is four types. Ceter et al. (2012) introduced five types of color and three types of seed shapes. Turki et al. (2013) introduced four types of color and three types of seed shape. Seed coat microsculpture types in medicagoid *Trigonella* species

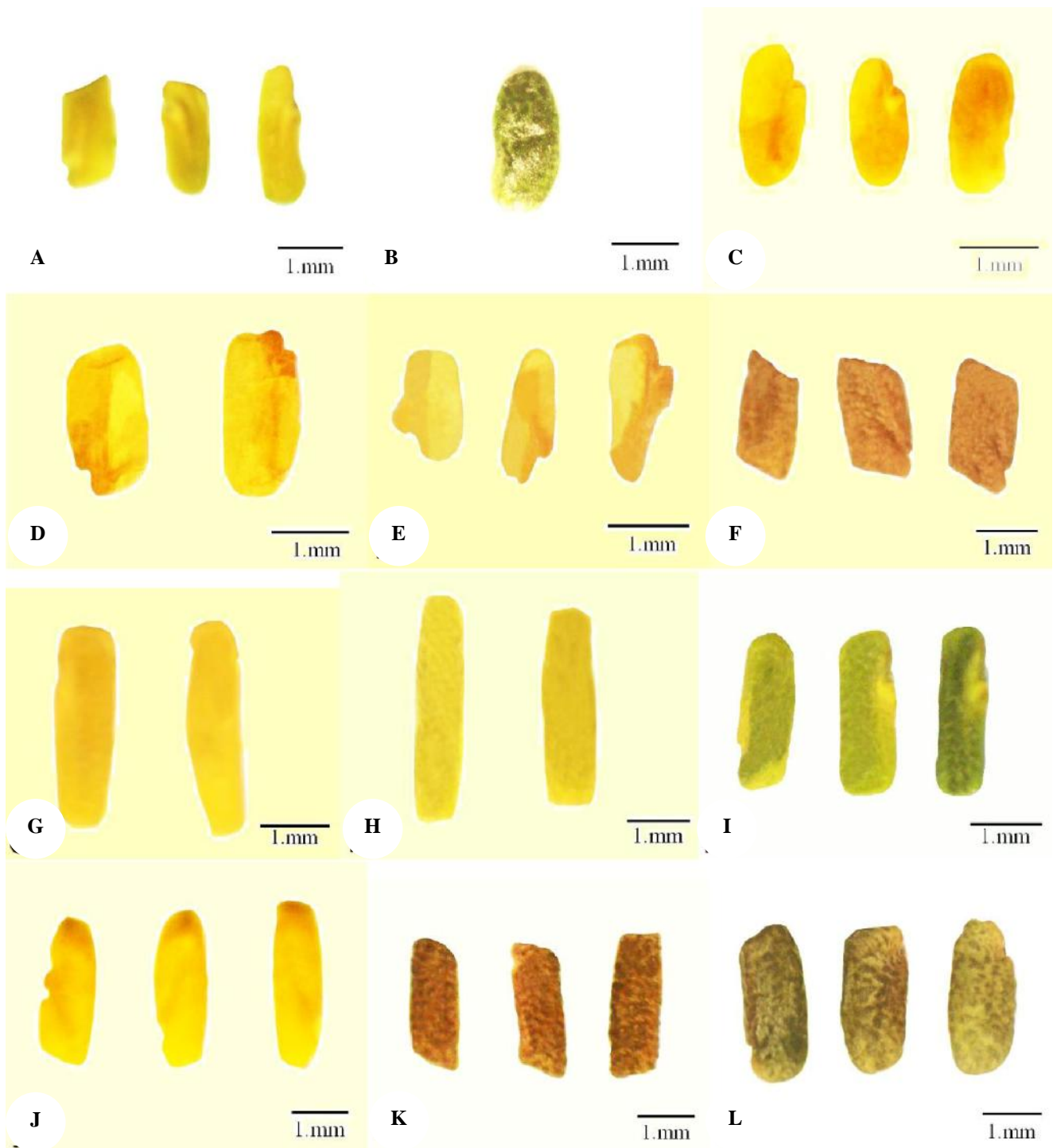


Figure 1. Stereomicroscope photograph of *Trigonella* seeds. A. *T. arcuata* (EA), B. *T. arcuata* (WA), C. *T. aurantiaca*, D. *T. crassipes*, E. *T. fischeriana*, F. *T. macroglochin*, G. *T. monantha* (EA), H. *T. monantha* subsp. *monantha* (WA), I. *T. orthoceras* (EA), J. *T. orthoceras* (WA), K. *T. persica*, L. *T. tenuis*.

were introduced by Small et al. (1990) as one type and by Ceter et al. (2012) as three types and Turki et al. (2013) as two types, while we observed two types, including reticulate and verrucate with secondary ornamentation (Table 3, Figures 3 and 4).

We introduce two 2 types of homogeneity cell size on seed surface and two types of hilum shapes in medicagoid

Trigonella species, while Ceter et al. (2012) recorded one type for each character and Turki et al. (2013) recognized two types of hilum shape and six types for cell size shape. In this study all morphological characters of Stereomicroscope and SEM Studies used to demonstrate phenetic relationship of medicagoid *Trigonella* species with the other taxa.



Figure 2. Stereomicroscope photograph of *Trigonella*, *Medicago* and *Melilotus* seeds. A. *T. coerulescens*, B. *T. calliceras*, C. *T. spruneriana*, D. *T. elliptica*, E. *T. uncata*, F. *T. brachycarpa*, G. *T. monspeliaca*, H. *T. turkmena*, I. *Medicago radiata*, J. *Melilotus officinalis*

Salimpour et al. (2013) showed that based on cluster analysis of morphological characters two major groups including *Medicago* and *Trigonella* (*Bucrates*) species join together. Turki et al. (2013) based on numerical analysis indicated that *Trigonella coerulescens* and *T. calliceras* (non medicagoid) are closely related and placed them at a farther distance from *T. monspeliaca*

(Medicagoid). In this study based on morphological seed characters mostly medicagoid *Trigonella* species are placed in a separate subcluster B1 and B2a (*Bucrates* species) but, non medicagoid species placed at a farther distance from the other species. *Medicago radiata* is placed at a separate cluster adjacent to *Bucrates* species, therefore our results agree with Salimpour et al. (2013) results.

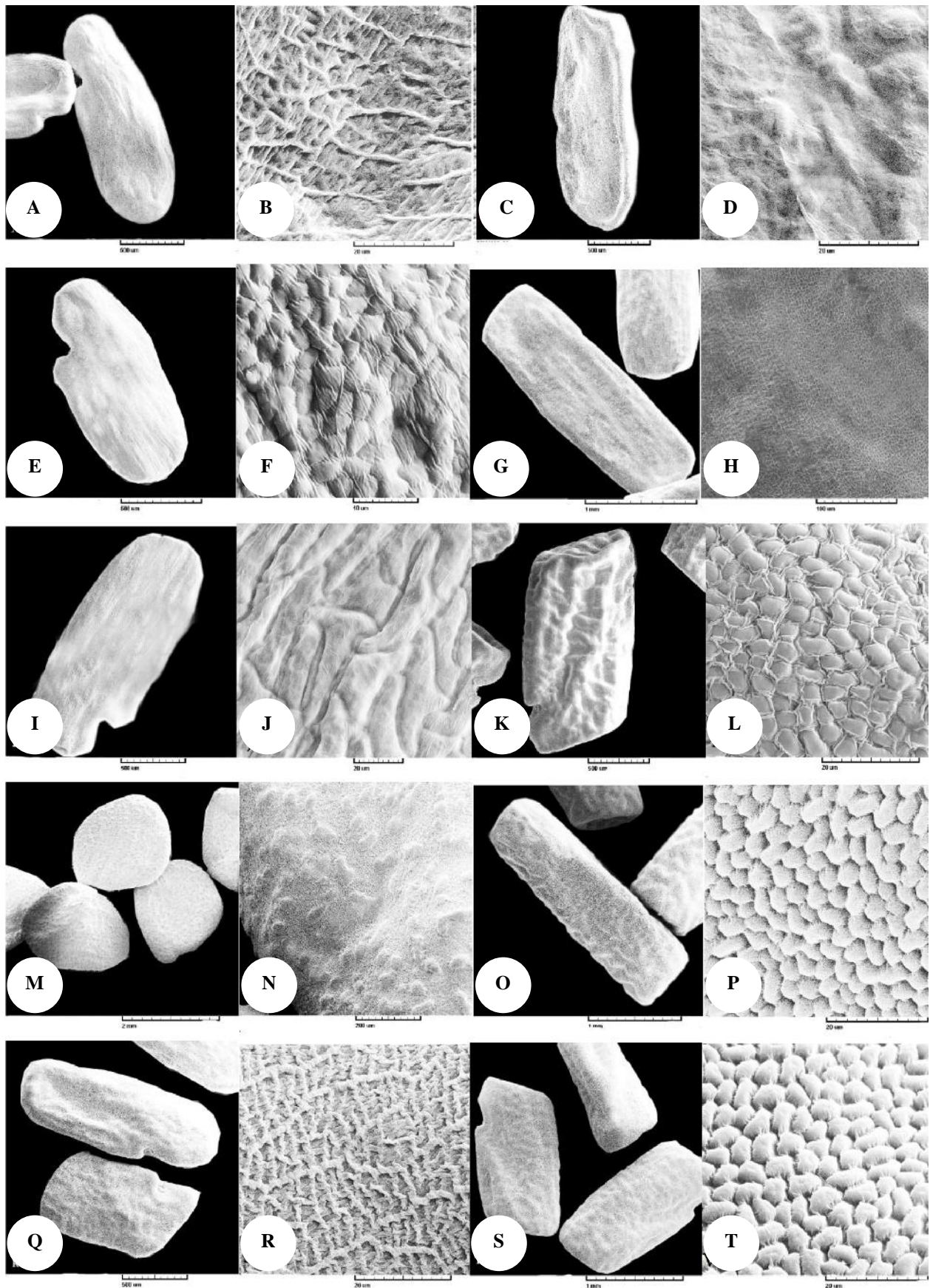


Figure 3. SEM microphotographs of *Trigonella* seeds. A-B. *T. arcuata* (EA), C-D. *T. arcuata* (WA), E-F. *T. aurantiaca*, G-H. *T. monantha*, I-J. *T. fischeriana*, K-L. *T. macroglchin*, M-N. *T. calliceras*, O-P. *T. monantha* subsp. *monantha*, Q-R. *T. orthoceras* (EA), S-T. *T. orthoceras* (WA).

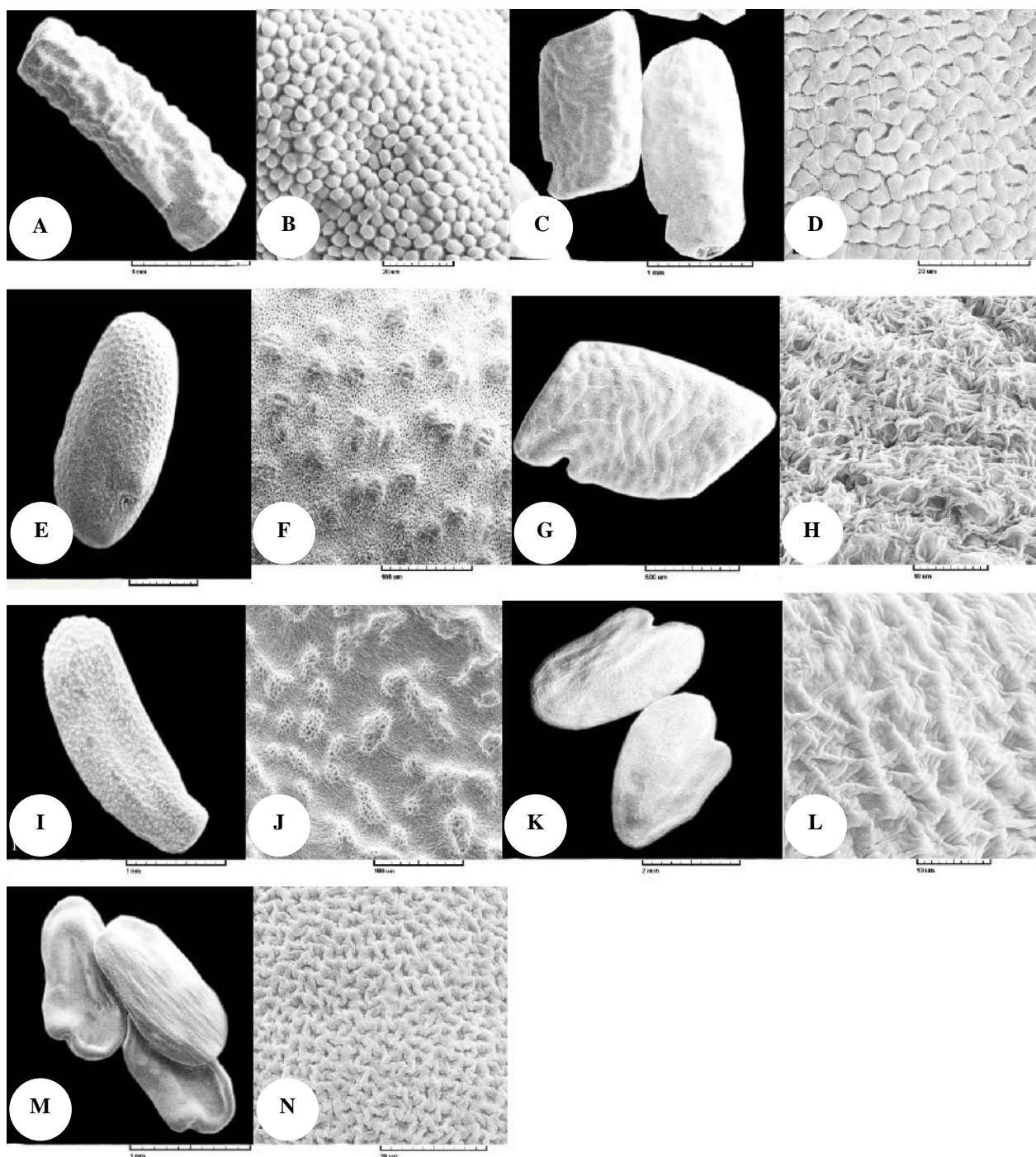


Figure 4. SEM Microphotographs of *Trigonella* seeds. A-B. *T. persica*, C-D. *T. tenuis*, E-F. *T. coerulescens*, G-H. *T. monspeliaca*., I-J. *T. spruneriana*, K-L. *T. elliptica*, M-N. *T. uncata*

According to Figures 5 and 6 most of medicagoid *Trigonella* species are placed in subclusters B1 (except *T. coerulescens*) and B2a (except *T. uncata*) and are more closely related to each other than the non medicagoid species. *T. coerulescens* and *T. calliceris* are placed in two separate groups, so this result does not agree with Turki et al. (2013) results, but agree with them because of these two non medicagoid species are placed at a farther distance from *T. monspeliaca* medicagoid species. In subcluster B1 majority of *Bucerates* species and *T. brachycarpa* from

Lunatae and in subcluster B2a two species from *Bucerates* section and *T. monspeliaca* from *Reflexae* section are nested together.

Result of PCA analysis showed that the first 3 components comprise about 63% of total variation. In the first component with about 26.3% of total variation, the seed shape, seed color, testa texture and hilum shape are the most variable characters ($0.61 < r > 0.68$). In the second factor with about 20.5% of testa variance, mean width and seed homogeneity are most variable characters

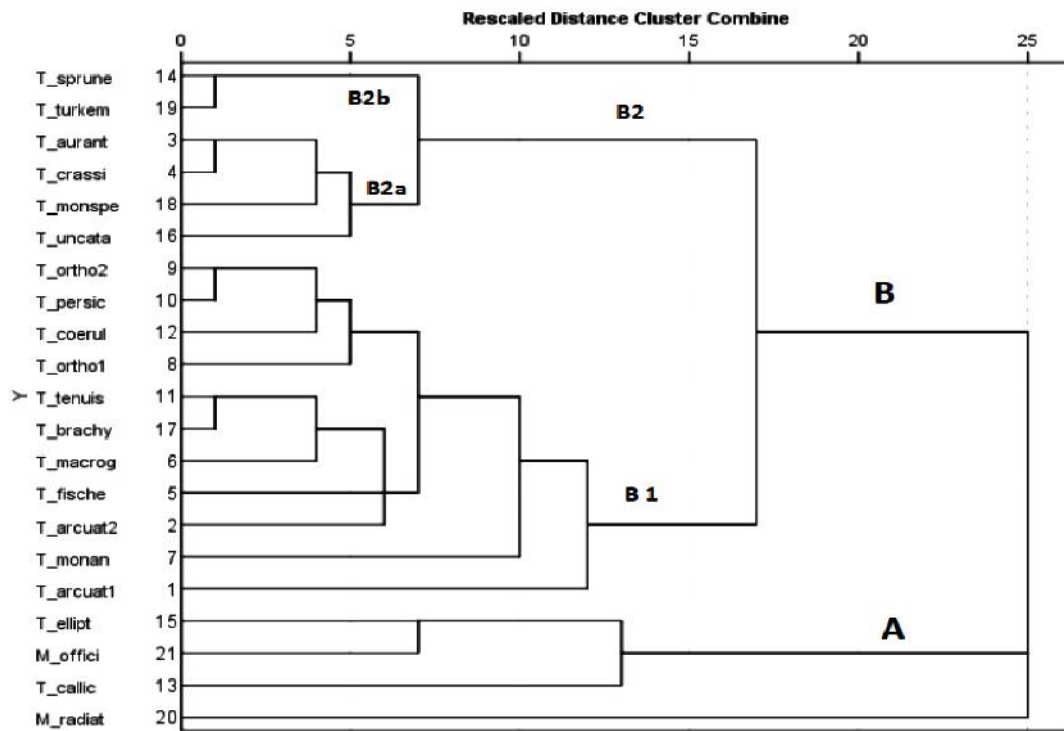


Figure 5. Phenogram based on morphological analyzing data of 22 taxa species (6 species of non medicagoid *Trigonella*, 11 species of medicagoid *Trigonella*, 1 species of *Medicago* and *Melilotus*). Note: T_arquat: *Trigonella arcuata*, T_aurant: *T. aurantiaca*, T_crassi: *T. crassipes*, T_fische: *T. fischeriana*, T_macrog: *T. macroglochis*, T_monan: *T. manantha*, T_ortho: *T. orthoceras*, T_persic: *T. persica*, T_coerul: *T. coerulescens*, T_callic: *T. calliceras*, T_ellipt: *T. elliptica*, T_brachy: *T. brachycarpa*, T_monspe: *T. monspeliaca*, T_turkem: *T. turkmena*, T_sprune: *T. spruneriana*, M_radiata: *Medicago radiata*, M_offici: *Melilotus officinalis*.

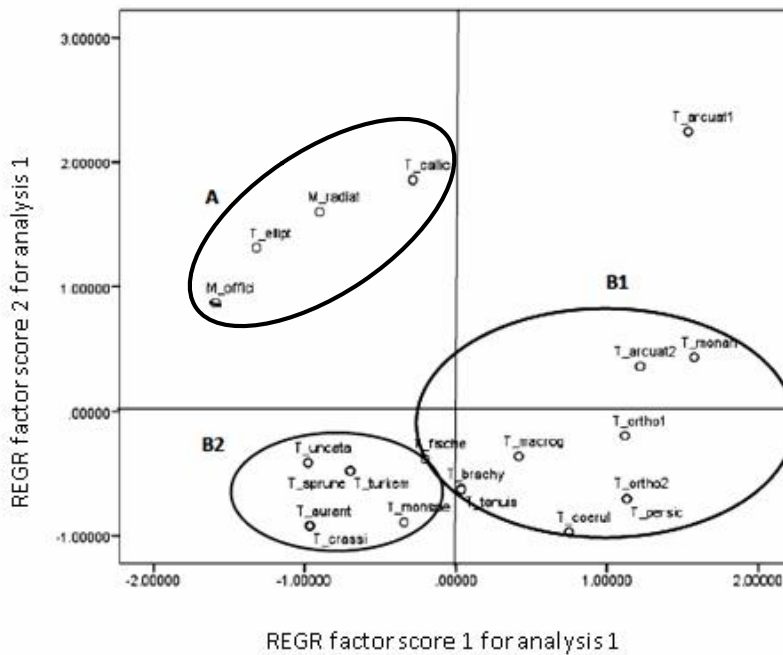


Figure 6. Relationship of all *Trigonella* species in Principal Component Analysis (PCA) based on seed morphological characters (abbreviations as Figure 5).

($0.66 > r > 0.68$). In the third factor with about 16.2% of total variance testa ornamentation is the most variable character ($r > 0.64$). *T. arcuata* and members of the group A, respectively, were diverse from members of the B1 and B2 groups by the most variable characters in the second factor. Also, the most variable characters in the first factor caused to separation of group B1 from B2. Result of PCA analysis confirmed phenogram result.

The results of this study agree with Flora Iranica (Rechinger 1984) which placed majority of medicagoid *Trigonella* species in *Bucerates* section, such as subclusters B1 and B2a based on seed morphology in our study. *T. monspeliaca* from *Reflexae* section and *T. brachycarpa* from *Lunatae* section grouped in medicagoid *Trigonella* species of *Bucerates* which is not in agreement of Flora Iranica treatment placing them in two separate sections from *Bucerates* section.

As a conclusion seed characters may be used as a useful tool in the taxonomy of the genus *Trigonella* but as Baum (1968) already pointed out more materials should be used in these studies.

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