International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 20-11-2019; Accepted: 21-12-2019
www.botanyjournals.com
Volume 5; Issue 1; January 2020; Page No. 109-113

Micromorphology of seeds of *Bucerates, Lunatae, Hymenocarpos* sections of *Medicago* L. species in Azerbaijan

Guvendiyev Vahid Musa[1*], Asgarov Aydin Musa[2]
[1, 2] Institute of Genetic Resources of ANAS, Department of Ecobotany and Taxonomy. Azerbaijan, Baku, Azerbaijan

Abstract
The micromorphological traits of seeds (*M. medicaginoides*, *M. monantha*, *M.orthocerac*, *M. monspeliaca*, *M. brachycarpa*, *M.radiata*) of six *Medicago* L. species spread in Azerbaijan have been studied with stereo microscope and scanning electron microscope (SEM). The status of some species of *Medicago* genus included to *Trigonellinae* of *Trifolieae* tribe is controversial. During the observation, the difference between the quality and quantity parameters of those taxa was revealed. The seeds have been compared for its shape, color, hilum shape, position and surface patterns and etc. Their taxonomical significance have been discussed. The studied traits are significant in determination of systematical position and identification of species.

Keywords: *Medicago, genus, micromorphological traits, scanning electron microscope*

Introduction

The modern geographic range of Medicago is the most phytogeographical regions of the world - Eurasia, Africa and the countries surrounding the Mediterranean (Sea). The genus Medicago is one of the largest genera-close to 83-87 species [29], of the tribe Trifolieae, subtribe Trigonellinae in the subfamily Faboideae. This subtribe grouped four genera Medicago, Melilotus, Trifolium and Trigonella [2, 5]. Many taxonomical studies have been carried out on ascertaining the relationship and the delimitation between Medicago and Trigonella. The studies tried to solve the problem by floral, seed and pollen morphology [26, 2]. The main taxonomic problem arises from these species known as "medicagoid" Trigonella, which exhibit flower and seed similarities with individuals of Medicago. These characters especially related to the explosive tripping pollination mechanism [27]. Some authors transfered these "medicagoid" 23 Trigonella species to genus Medicago [27, 28, 25, 4, 2, 20]. A molecular phylogeny supported the transfer of the medicagoid species from the genus Trigonella to Medicago (24). Analyses of nrDNA ITS and ETS and plastid trnK/matK and nuclear GA3ox1 data supports inclusion of the medicagoid Trigonella species in the genus Medicago [30, 31].

According to "Flora of USSR" there are 36 Medicago L. species [9], 30 of which were revealed in Caucasus [19]. According to Flora of Azerbaijan 20 species of alfalfa were revealed in Azerbaijan territory [8]. Based on the latest data 17 species and 4 subspecies of the genus are spread in Azerbaijan [15].

From 23 transferred species, 6 species of Trigonella genus spread in Azerbaijan were included into Medicago. Numerous taxonomical researches have been carried out on the Medicago L. species and they were classified into 12 sections [29]. Medicago L. species, spread in Azerbaijan, were classified into 7 sections (Table 1).

Table 1: Division of Medicago L. species spread in Azerbaijan by sections

№	Sections	Species
1	Sec. Medicago	M. sativa, M. papillosa
2	Sec. Spirocarpos	M. littoralis, M. rigidula, M.truncatula, M. arabica, M. minima, M. polymorpha, M. meyeri
3	Sec. Orbiculares	M.orbicularis
4	Sec.Hymenocarpos	M. radiata
5	Sec. Lunate	M. brachycarpha
6	Sec. Lupularia	M. lupulina
7	Sec. Buceras	M. medicaginoides, M. monantha, M. monspeliaca, M.orthocerac

Study of micromorphological traits of external surface of the seed provided a new approach for the identification of the systematical relations between Trigonella and Medicago L. species [25, 14, 32, 18].

Implementation of such researches figured on clearing up to positions of some taxa with controversial systematic status. In numerous researches, micro morphological traits were used in order to identify some taxa of leguminous family and systematic classification [13, 35, 6, 23].

Description of general shape of the seed is characterized mainly by two criteria - width and length sizes. The seeds are primarily of different types: eggshaped, obovate, pearshaped, ovate, ellipse-shaped, kidney-shaped, heart-shaped, lanceolate etc. Microstructure of seed surfaces are wrinkled, cracked, plane, rough, with ravines, hairly, holed, netshaped veined and etc. The aim of this study is to evaluate seed morphological characters in the taxonomy of the genus Medicago, especially medicagoid species.

Material and Methods

Seed materials have been collected from different populations (in situ conditions) in Azerbaijan territory. Collected seed materials were filled into special sterile paper bags and dried with silicagel in laboratory conditions. Colour, length and width size of seeds have been determined by Leica EZ4D stereomicroscope with 0, 1 mm accuracy. In order to study in electronic microscope the seeds were put into the stabs with adhesive paper and covered with gold pollen by JOEL JFC1100E ion-sputtering device. Micro morphological observations in the microstructure of seed material surface were carried out by JEOL JSM 6060 electronic microscope (SEM), the photos were taken and spectral analyses of distinct places was carried out. Measures have been carried out on minimum 20 seeds per population. The data on collection sites of species was given in Table 1.

In order to accurate some of terms Lersten (1982), Small *et al*. [25], Gupta [14], Pinar *et al*. [23], Gunes *et al*. [12], Gunes [11], Teixeiraet *et al*. [33], Ozbek *et al*. [22] and Mirzaei *et al*. [21], Bojňanský and Fargašová [3] were used. Simpson and Roe graphical test has been used for statistical calculations [34].

Table 2: Data on collection sites of investigated of *Medicago* L. species

Species	Description of Location	Area Sampled (m^2)	Lat./ Long.	S.L.R. (m)
M.medicaginoides	Dist. Lerik, vill. Mistan	100<	N 38°38.366' E 048°26.967'	1896
M.monantha	Dist. Lerik, vill. Diviagac	50-100	N 38°65.123' E 048°41.369'	1392
M.orthoceras	Dist. Lerik, vill. Xelife	50<	N 40°37.824' E 048°01.538'	1714
M.monspeliaca	Dist. Absheron, vill. Mehemmedi	100<	N 40°29.973' E 049°53.585'	34
M.brachycarpa	Dist. Shahbuz territory hayfield area	100<	N 39°32.354' E 045°49.380'	2256
M.radiata	Dist. Ordubad, vill. Bash Dizcak	50-100	N 38°59.029' E 045°48.413'	887

Results and Discussion

For their morphological traits, some investigated taxa differed slightly from each other. Morphology of seed surface did not change and had stabile shape. The seeds were slightly effected by external ecological factors [7]. The microstructure of seed surface of each specie has spesific micro morphological traits, which is taxonomically significant in identification of their status and for their determination. Investigation of numerous crop groups shows that seed morphology and anatomical features are the most taxonomocally conservative and indirect morphological traits [35, 10, 16]. Study of ultrastructure of seed surface with electronic microscope (SEM) is known as a reliable approach-research method for evaluation of phylogenetic relations of species and identification of taxon. This factor sometimes is considered as a specific trait for genus [1]. Based on observations carried out by the light and electronic microscope (SEM) the diversity in quality and quantity parameters was recorded in investigated seeds of Medicago species.

Seed size

The seed size in Medicago was different (Table 1). For their length and width the seeds were classified into three groups: I. L: 1.5 - 1.9 mm, W: 0.5 - 1 mm; II. L: 2.0 and 2.9 mm, W: 1mm and 2.0; III. L: 3 mm and 3≤; W: between 2 mm and 3≤ mm. Those two characters are strongly positively correlated. This indicates the strong positive correlation between two traits (0.72, $p < 0.047$).

The largest seeds were revealed in M. brachycarpa (average length 2.25 mm and 2.04 width) and M. radiata (average length 2.03 and 2, 04 width). The smallest seeds were revealed in M. monantha (average lehgth 1.86 mm and 0.77 width) and M.orthocerac (average length 1.86 and 0.72 mm width).

Table 3: Trait indicators of seeds of Medicago L. species

N⁰	Sectiae, species	Length (µm)			Width (µm)			Weight (1000 st./g)	Outline	Colour	Testa appearance		
		Min	Max	Mean	Min	Max	Mean				Sculpture (Ornamentation)	Hilum-Micropylar region	
												Shape/ Colour	Sizes (µm)
1	Sec.Bucerates *M. medicaginoides*	1.75	2.5	2.02	0.5	1	0.78	0.92	Oblong	Green	Ridged-Tuberculate-Verrucate	Elliptic- white	0.11
2	*M. monantha*	1.5	2.5	1.86	0.5	1	0.77	1.02	Oblong	Green	Ridged-Tuberculate-Verrucate	Elliptic- white	0.12
3	*M.orthocerac*	1.5	2.5	1.86	0.5	1	0.72	0.93	Oblong	Green	Ridged-Tuberculate-Verrucate	Elliptic- white	0.12
4	*M. monspeliaca*	1.5	3	1.88	0.5	1.5	1.05	0.67	Squara-elliptic	Yellow	Ridged-Tuberculate-Verrucate	Elliptic- white	0.15
5	*Sec.Lunatae M. brachycarpa*	2	3	2.23	1.5	2.5	2.04	2.43	Ovoid	Dark yellow	Verrucate	Elliptic- white	0.16 ±0.04
6	*Sec.Hymenocarpos M. radiata*	1.5	2.5	2.03	1.5	2.5	2.04	0.97	Elliptic	Dark yellow	Verrucate	Elliptic- white	0.15 ±0.01

Seed weight changed between 0.001 and 0.091 mg averagely. The heaviest and the largest seed was in M. brachycarpa (2.23 mg), while the most lightweight seed was noted in M. monspeliaca (0.001 mg).

Seed shape and color

Seeds weare ovoid, elliptic, oblong, allantoid, allantoid-oblong, square-elliptic, sferoidal, elongated four cornered shapes. Various changes in seed shape of *Medicago* L.

species have been indicated. In general, three major shapes of hilum-elliptic and rarely elongated or ovate shapes were found. Hilum colors were white. Hilum size changed between 0, 1 with 0, 2 mm.

Seed coat surface

Some different ornament shapes were observed in structure segments of seed surface. Microstructure features of atoms of investigated taxa were given in Table 3.

The changes in seed micromorphology of Medicago L. species were revealed generally in seed weight, shape, color, and seed coat structure and hilum morphology. Seed coat ornamentation saved its stable shape without changes. Seeds were exposed to external environmental factors more than other organs [7].

The final results obtained using light and electron microscope (SEM) were presented in Table 3 and Figures 1 and 2. The microstructure of the seed surface of each species has specific micromorphological features, and is of taxonomic significance in the determination of species status and their identification. The study of many plant groups shows that the morphology and anatomical features of the seeds are quite conservative and indirectly taxonomically important morphological traits [35, 10, 16]. Tai has recorded that the seeds of Trigonella have changable micromorphological features that can be used for taxonomic purposes [32].

Different morphological traits in Medicago L. species has shown that taxonomical use of these traits could be a taxonomic method of identifying certain taxa. Among the studied traits, there is a positive correlation between the length and width of the seed.

Seeds of Hymenocarpos L. section species showed the smallest difference (2.03 to 2.04 mm) between these sections. M. brachycarpa species of the Lunatae section have larger seeds (ranging from 2 to 3 mm length). There is considerable difference in seed shape and color of the other studied species (Table 3, Figure 1). Investigations carried out by by Gupta (1991) and Taia (2004) revealed differences in the shape and color of Trigonella species. The color of the hilum is white in all taxa [14, 32].

The structure of the cut piece of seeds of the Medicago consists of the epidermis-Malpigi layer, the hypodermis (lymphotic layer), the internal layer (parenchyma), the endosperm, and the embryo [25, 17]. Although this structure is characteristic for many families, it cannot be concerned all genera of Leguminous [13, 35, 6]. Based on the patterns, shape and color of the seeds surface of the studied species, we classified these three Medicago L. sections into two main type-groups.

Type I: seeds, with wrinkled-twisted surface. These include the Hymenocarpos and Lunatae sections, which have the same surface patterns due to the microstructural features. The seeds of the Hymenocarpos section are elliptic, and the seeds of the Lunatae section are ovate.

Type II: Seeds with splitted, toothed, and circular swollen surface: This type of traits has been observed in Bucerates section species. Species belonging to both groups can be distinguished by color and shape (Table 3, Figure 1).

Another investigation carried out by Guvandiyev and Askerov, revealed the differences in the shape and color of Medicago, Orbiculares, Lupularia, and Spirocarpos sections of the Medicago L. spread in Azerbaijan [15]. Circular swollen, incurvate patterns observed on the surface of the species of Bucerates section were also found in the M. orbicularis species of the monotypic Orbiculares section.

Conclusion

This investigation showed that there are great differences among the seed surface microstructure, shape and color of the three sections of the Medicago L. species. However, these features of the seeds did not support the subgenus classification, and the revealed traits did not provide significant information that could be used to differentiate the sections of this genus. However, a combination of data on unique micromorphological features of the seeds surface of the studied species will facilitate their identification.

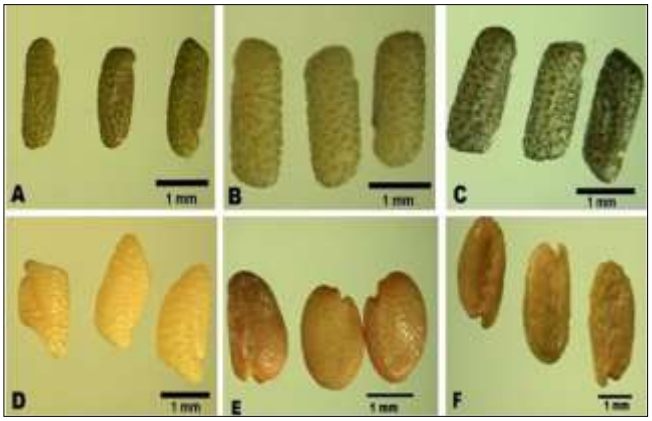

Fig 1: Stereo Microphotograph of *Medicago* L. seeds. A; M. medicaginoides, B; M. monantha, C; M. orthoceras, D; M. monspeliaca, E; M. brachycarpa, F; M. Radiata

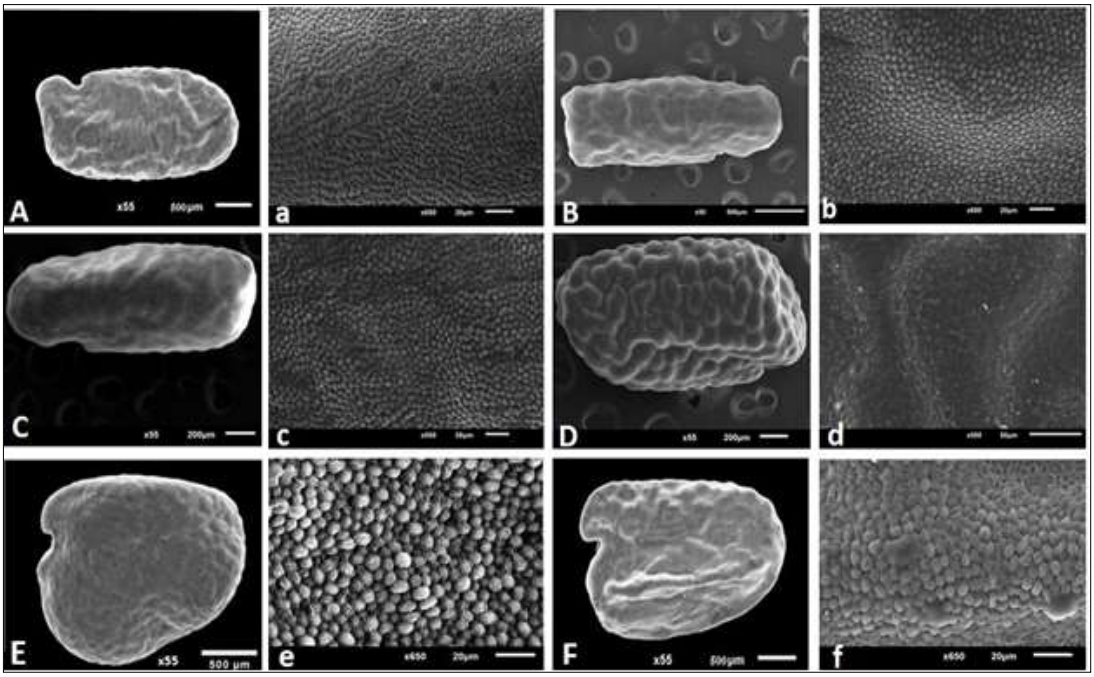

Fig 2: SEM Microphotograph of Medicago L. seeds. A, A; M. medicaginoides, B, b; M. monantha, C,c; M. orthoceras, D, d; M. monspeliaca, E, e; M. brachycarpa, F, f; M. radiata

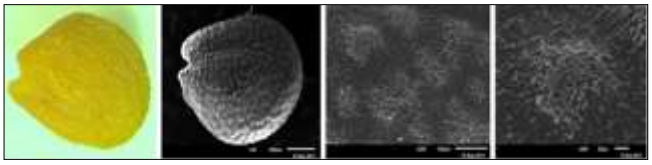

Fig 3: Seed image of M. orbicularis species in stereo and electron microscope (SEM)

Acknowledgements

Part of the research was carried out on an electron microscope (SEM) at ANAS Geology and Geophysics Institute. We would like to thank for ANAS Institute of Geology and Geophysics employee of the Department of Micro Paleontology Shafag Bayramova.

References

1. Barthlott W. Epidermal seed surface characters of plants systematic applicability and some evolutionary aspects. Nord. J Bot, 1981; 1:345-355.
2. Bena G. Molecular phylogeny supports the morphologically based taxonomic transfer of the “Medicagoid” Trigonella species to the genus Medicago L. Plant Syst Evol, 2001; 229:217-236.
3. Bojňanský V, Fargašová A. "Atlas of Seeds and Fruits of Central and East-European Flora, Amstherdam, Netherlands; Springer, 2017, pp. 1046
4. Boulos L. Flora of Egypt. Volume 1 (Azollaceae to Oxalidaceae). Al-Hadara Publishing, Cairo, Egypt, 1999.
5. Dangi RS, Lagu MD, Choudhary LB, Ranjekar PK, Gupta VS. Assessment of genetic diversity in Trigonella foenum-graecum and Trigonella caerulea using ISSR and RAPD markers. Plant Biology. 2004; 4:13-16.
6. Fawzi NM. Macro- and micromorphological seed characteristics of some selected species of Leguminosae. Research Journal of Botany. 2011; 6:68-77.
7. Fayed AA, Hassan N. Systematic significance of the seed morphology and seed coat sculpture of the *Genus euphorbia* L. (Euphorbiaceae) in Egypt. FL Medit. 2007; 17:47-64.
8. Flora of Azerbaijan. Baku: AC Azerbaijan SSR, vol. V, p. 248-265.
9. Flora of SSSR, vol. XI., Leningrad, 1945, p. 129-176.
10. Gabr DG. Seed morphology and seed coat anatomy of some species of Apocynaceae and Asclepiadaceae. Ann. Agric. Sci, 2014; 59:229-238.
11. Gunes F. Seed characteristics and textures of Pratensis, Orobon, Lathyrus, Orobastrum and Cicercula srctions from Lathyrus (Fabaceae) in Turkey. Plant Syst Evol. 2013; 299:1935-1953
12. Gunes F, Cirpici A. Seed Characteristics and Testa Textures some Taxa of *Genus lathyrus* L. (Fabaceae) from Turkey. Int J Agric Biol. 2011; 13(6):1814 9596.
13. Gunn CR. Seed topography in the Fabaceae. Seed Sci. Technol. 1981; 9:737-757.
14. Gupta M. Seed coat structure in some species of Trigonella. Scanning Microsc. 1991; 5(3):787-796.
15. Guvendiyev VM, Askerov AM. Taxonomy of species of the genus of Medicago L. (Fabaceae Lindl.) Flora of Azerbaijan and micromorphological traits of seeds of some species. RF Krasnoyarsk, Bulletein of KSAU. 2019; 1(142):192-200.
16. Heneidak S, Abdel-Khalik K. Seed coat diversity in some tribes of Cucurbitaceae: implications for taxonomy and species identification. Acta Bot. Bras, 2015; 29:129-142.
17. Jha SS, Aruna P. Seed Structure in Medicago L. Flora 187: pp. 209-218 Lucknow, İndia, 1992.
18. Khandani S, Assadi M, Nejatsatari T, Mehregan I. Pheneticanalysis of the genera medicagoid Trigonella, Medicago and Melilotus (Fabaceae) on seed coat in Iran. Biodiversitas. 2016; 17(1):162-171.
19. Lachashvili IY. "Medicago of the Caucasus". Monograph. Tiflis, 1967.

20. Marzouk RM, EL-Bakatoushi R. Assessment of relocation of Trigonella cylindraceae L. and T.polyceratia (L.) trautv. To genus Medicago as inferred by RAPD and RFLP analyses. Pakistan Journal of Botany. 2011; 43(5):2289-2294.
21. Mirzaei L, Assadi M, Nejadsatari T, Mehregan I. Comparative seed and leaf micromorphology of Colutea species (Fabaceae) from Iran. Environ Exp Biol, 2015b; 13:183-187.
22. Ozbek F, Ozbek MO, Ekici M. Morphological, anatomical, pollen and seed morphological properties of Melilotus bicolour Boiss & Balansa (Fabaeae) endemic to Turkey. Aust J Crop Sci. 2014; 8(4):543-549.
23. Pinar MN, Duran A, Ceter T, Tug N. Pollen and seed Morphologyof the Genus Hesperis L. (Brassicaceae) in Turkey. Turk J Bot, 2009, 83-96.
24. Ranjbar M, Hajmoradi Z. Notes on Medicago sec. Lunatea Boiss. And Trigonella sec. Bucerates Boiss.of the tribe Trifolieae (Fabaceae) with two new records from Iran. Iran J Bot. 2012; 18(2):235-238.
25. Small E, Brookes B, Lassen P. Circumscription of the genus Medicago (Leguminosae) by seed characters. Can. J Bot, 1989; 68:613-629.
26. Small E. Pollen-ovule pattern in tribe Trifolieae (Leguminosae). Plant Systematics and Evolution, 1986; 160:195-205.
27. Small E. A taxonomic study of the "medicagoid" Trigonella. Can. J Bot, 1987; 65:1199-1211.
28. Small E. Generic changes in Trifolieae subtribe Trigonellinae. In: Stirton C. H. (ed) Advances in legume systematic. Part 3, Vol. 3. Royal Botanic Gardens, Kew, 1987b, 169-181.
29. Small E. "Alfalfa and Relatives: Evolution and Classification of Medicago". Canada, Ottawa,pp. 737
30. Steele KP, Wojciechowski MF. Phylogenetic analyses of tribes Trifolieae and Vicieae, based on sequences of the Plastid gene matk (Papilionoideae: Leguminosae). Advances in Legume Systematics, part 10, Higher Level Systematics. Royal Botanic Gardens, Kew, 2003-2011.
31. Steele KP, Ickert-Bond SM, Zarre S, Wojciechowski MF. Phylogeny and character evolution in Medicago (Leguminosae): Evidence from analyses of plastid trnK/matK and nuclear GA3ox1 sequences. Am. J. Bot. 2010; 97(7):1142-1155.
32. Tia WK. Tribe Trifolieae: Evidence from seed characters. Pakistan J Biol. Sci. 2004; 7(7):1287-1302.
33. Teixeira de QR, Azevedo de TAM, Lewis GP. Seed morphology: anaddition to the taxonomy of Tephrosia (Leguminoseae, Paplionoideae, Millettieae) from South America. Plant Syst Evol, 2013; 299:459-470.
34. Van der Pluym A, Hideux M. Application's d'une methodologie quantitative ala palynologie d'Eryngium maritimum (Umbelliferae). Plant Syst. Evol, 1997; 127:55-85.
35. Zoric L, Merkulov L, Lukovic J, Boza P. Comparative seed morphology of Trifolium L. species (Fabaceae). Period Biol, 2010; 112:263-272.