



Taxonomic Relevance of Seed and Seedling Morphology in Some *Medicago* (Leguminosae) Species

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SEED and seedling morphology of 25 taxa from two sections and three subsections of the genus *Medicago* (Leguminosae-Papilionoideae) were studied using fresh materials. Seed, hypocotyl, paracotyledon, epicotyl and first leaf characters were used to construct an artificial key for identification purposes. Seed color and shape, radical length in relation to seed length, epicotyl color and hairiness, first foliage leaf shape and hairiness were all used as morphological descriptors for species identification. Cluster analysis was used to determine taxonomic interrelationships among the investigated taxa. The *Spirocarpos* and *Medicago* sections of *Medicago* L. were clearly distinguishable, whereas other sister taxa largely grouped together.

Keywords: Embryo, Leguminosae, *Medicago*, seed, Seedling morphology, Taxonomy.

Introduction

The genus *Medicago* L. comprises approximately 87 species of herbs and shrubs and are widespread from the Mediterranean to Central Asia; including the legume model species *M. truncatula* and the widely cultivated forage crop and weedy species *M. sativa* (Steele et al., 2010). EL-Ramady et al. (2020) considered *M. sativa* crop as a sustainable solution for the conflict of food and plant-based resources.

Taxonomically, *Medicago* L. belongs to the tribe Trifolieae of the Leguminosae-Papilionoideae subfamily (Steele et al., 2010). The Leguminosae have been extensively investigated in various disciplines, e.g., anatomy (Lackey, 1978; Pernía & Melandri, 2006; Evans et al., 2006), palynology (Erdtman, 1945; Ferguson 1984; Pupuleku et al., 2012), chemotaxonomy (Harborne et al., 1971; Hegnauer, 1994), phylogeny (Doyle et al., 2000) and embryology (Bryans & Smith, 1985; Hughes et al., 1997).

Juvenile stages of plants are often so different

from adult stage that it is hard to correlate the seedlings with an adult plant even with good field knowledge (Ahammed & Paria, 1996). Seedling characters are as important and reliable as those of the flowers and fruits present at the adult stage in the delimitation of species and genera (Paria et al., 2010). Knowledge of seedling features provides taxonomical as well as biodiversity information and helps in many other aspects such as identification of plants at early growth stages, hypocotyl tissue culture, viability tests of seeds, crop management and forestry (Sanyal & Paria, 2015; Kumar & Devendra, 2016). In the Leguminosae, seed and seedling morphology studies have provided characters with taxonomic relevance (e.g. Gunn, 1981, 1984; Gurgel et al., 2012).

A. De Candolle (1825) uses the shape of embryo axis, plumule and radicle as well as the position of the radicle relative to the cotyledons as diagnostic characters of subfamilies within the Leguminosae. He divides this large family into two unequal groups and names the subfamilies according to the shape of embryo axis. The Papilionoideae with curved embryo axis are named Curvembriae, and

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both the Caesalpinioideae and Mimosoideae with straight embryo axes are named Rectembriae.

Urban (1873) was the first to prepare a comprehensive analysis of the genus *Medicago* and noted the presence of a swelling “joint” (pulvinus) at the bases of cotyledons which he called cotyledonary pulvini. He found that cotyledonary pulvini are absent in every species of *Medicago* he examined, and present in species of the sister genera *Trigonella* and *Melilotus* and many species of *Trifolium*. Pulvini are joints at the base of leaves (usually leaf blades) that control leaf movement. *Medicago* leaves are trifoliolate and have pulvini or pulvinuli at the base of each leaflet. However, pulvini at the base of cotyledonary are only present in two *Medicago* sections (Lunatae and Buceras) and in the closely related genera *Trigonella* and *Melilotus* (Small, 2010).

Fernando et al. (1999) established the importance of phenetic analysis during the characterization of three woody *Medicago* species belonging to sect. *Dendrotelis* using 20 morphometric characters of seedlings and provided keys for species identification. Marisol Lo Bianco et al. (2015) described seed size, shape, color and texture for three *Medicago* taxa of sect. *Dendrotelis*. The studied species were perfectly distinguishable, confirming the current taxonomic treatment at the section level.

Turki et al. (2016) describe and characterize the macro- and micro-morphological characters of seedlings for 29 *Medicago* species. The study shows that seedling characters (cotyledonary leaf shape, area, blotches, pulvini, first foliage leaf area, and number of leaflets, shape, blotches, apex, margin, base, hairiness, hair types, and stipule margin) are taxonomically significant at the sectional level. Cotyledonary pulvinus characters distinguished species from the *Lupularia* and *Buceras* sections from species of other sections. Also, seedling characters were sufficient to enable the construction of an artificial key for identification of the 29 *Medicago* species studied.

Within *Medicago*, gross morphological features of the fully grown plants are so similar that: (i) The taxonomic placement of most taxa is highly controversial and nomenclature is so confused that some taxa have more than 20 synonyms each in the database of the Global Biodiversity Information Facility (<https://www.gbif.org>), and

(ii) The identification of species and varieties cannot be performed with any degree of certainty. Therefore, the present study was undertaken in search of another source of reliable characters to resolve these two issues by accumulating additional information about seed, embryo and seedling morphology and to apply such data to the elucidation of the taxonomic relationships within the genus and to the identification of various taxa at the early stages of their growth.

Materials and Methods

Seeds of 25 taxa of *Medicago* were obtained from the Institute of Plant Genetics and Crop Plant Research (IPK) in Germany and the International Center for Agricultural Research in the Dry Areas (ICARDA) in Syria; their origins and accession numbers are listed in Table 1. Seeds were stored in plastic bags and conditioned in a refrigerator at 5°C until their use. The extremely intricate nomenclature of *Medicago* species and notho-taxa was carefully updated mainly with the help of The Plantlist.org (2020), the Global Biodiversity Information Facility (2021), and Tropicos.org (2021). The few names not found in these databases were resolved using other sources such as Heyn (1963), IPNI (2020), and the African Plants Database (2020).

The following external seed characteristics were assessed: color, shape, size, radical length relative to seed length and hilum shape and size. Terminologies in the works of Sanyal & Paria (2015), Kumar & Devandra (2016), and Chanda *et al.* (2018) were used as references for descriptions of intact seeds. To observe internal characteristics, ten seeds were scarified by soaking in 100% sulfuric acid for 20 min to soften the hard seed coat (Ortega-Olivencia & Devesa, 1997) and then washed three times in water and the seed coat was removed. For every species, 50 seeds were germinated on wet filter paper in a Petri dish for four days.

Twenty-five pre-germinated seeds of each species reaching a radicle length of 1-3cm were planted at 1cm depth in five plastic pots (10cm in diameter and 15 cm depth) filled with soil (sand and peat moss, 2:1, respectively). Irrigation was carried out with tap water. Gross morphological variations were recorded directly from fresh specimens, which were then dried, preserved and kept as herbarium specimens in the Herbarium of the Botany and Microbiology Department, Faculty of Science, Menoufia University (MUH).

TABLE 1. List of 25 *Medicago* species and varieties referred to by section and subsection according to Lesins & Lesins (1979), together with their origins and sources of seeds

Sections	Subsections	Taxon No	Species	Varieties	Source & accession number	Origin
Medicago	Falcago	1	<i>M. sativa</i> L.		IPK /1771588	Deutchland
		2	<i>M. glomerata</i> Balb.		IPK/1772029	Unknown
		3	<i>M. sativa</i> L. subsp. <i>hemicycla</i> (<i>Grossh.</i>) <i>C.R.Gunn</i>		IPK/1771973	Unknown
		4	<i>M. falcata</i> L.		IPK/1772209	Unknown
		5	<i>M. sativa</i> L. subsp. <i>varia</i> (Martyn) Arcang.		IPK/1771590	Germany
		6	<i>M. polychroa</i> Grossh.		IPK/1773054	Soviet Union
	Suffruticosae	7	<i>M. suffruticosa</i> DC.		IPK/1772161	Unknown
	Intertextae	8	<i>M. intertexta</i> (L.) Mill.	<i>intertexta</i> *	ICARDA/109204	Morocco
		9		<i>ciliaris</i> (L.) Heyn.*	ICARDA/59039	Turkey
	Rotatae	10	<i>M. rotata</i> Boiss.	<i>rotata</i>	ICARDA/55408	Jordan
		11		<i>littoralis</i>	ICARDA/53502	Syrian A.R.
	Spirocarpos	12	<i>M. littoralis</i> Loisel. <i>M. rigidula</i> Bourg. ex Nyman (in Tropicos.org), (= <i>M. rigidula</i> (L.) All.; in The Plantlist)	<i>inermis</i> (Moris) Arcang.	ICARDA/110936	Libyan A. J.
		13		<i>rigidula</i>	ICARDA/58251	Iran
		14		<i>agrestis</i> Burniat***	ICARDA/53582	Syrian A.R.
		15		<i>cinerascens</i> (Jord.) Ponert***	ICARDA/56022	Jordan
		16		<i>submitis</i> (Boiss.) Ponert***	ICARDA/53375	Jordan
		17		<i>truncatula</i>	ICARDA/53096	Morocco
	Pachyspireae	18	<i>M. truncatula</i> Gaertn.	<i>inermis</i> Moris	ICARDA/110934	Libyan A. J.
		19		<i>tricycla</i> (Nègre) Heyn	ICARDA/110943	Libyan A. J.
		20		<i>xlittoralis</i> (Rohde ex Loisel.) Ponert	ICARDA/55602	Morocco
		21	<i>M. murex</i> Willd.	<i>murex</i>	IPK/1771978	Greece
		22	<i>M. aculeata</i> Gaertn.**	<i>aculeata</i>	ICARDA/ 55885	Syrian A.R.
		23	<i>M. doliata</i> Carmign.	var. <i>doliata</i>	ICARDA/ 55137	Syrian A.R.
		24	<i>M. tornata</i> (L.) Mill.	<i>lenticularis</i> (Ders.) Nègre	ICARDA/ 111099	Tunisia
		25		<i>lenticularis</i> f. <i>aculeata</i> Urb.	ICARDA/ 53020	Palestine

ICARDA= International Center for Agricultural Research in the Dry Areas (ICARDA); IPK= Institute of Plant Genetics and Crop Plant Research.

*= Name accepted in IPNI (2020); **= Name accepted in African Plant Database (2020); *** = Treated as synonyms of var. *rigidula* in the GR Database of the National Genebank of Tunisia.

Saplings were evaluated until the complete expansion of the third eophyll, as done for other Leguminosae species in the studies of Rodrigues & Tozzi (2008) and Rodrigues et al. (2012). Seedlings at different stages of growth and development were collected and photographed with a digital camera. Cotyledonary and first leaf area were determined by the millimeter graph paper method as in Pandey & Singh (2011). Descriptive terminology of seedlings follows Duke (1965), Burger (1972), Hickey (1973), Dilcher (1974), and Vogel (1980). Gross morphological features (e.g., venation and hairiness) of the seedlings were observed using a binocular microscope (Wild M-3 Stereomicroscope).

An artificial key was constructed based on the diagnostic seedling characters. For numerical analysis, the characters and their states listed in Table 2 were coded and scored in a data matrix. A dendrogram was constructed on the basis of 65 morphological characters of seeds, embryo and seedlings listed in Table 3 to illustrate the hierarchical relationship between the studied *Medicago* species using the SPSS statistics software program version 16.0 (SPSS Inc., Chicago, IL).

Results

Seed shape was subreniform in all taxa except in three species of section *Medicago* that were distinguished by mitaform seeds (*M. glomerata*, *M. sativa* subsp. *hemicycla* and *M. suffruticosa*). Blackish brown testa distinguished the two varieties of *M. intertextata* from all other taxa with yellow-dark brown testa. Hilum shape was circular in all studied species of section *Medicago* and varied from elliptic to circular in the taxa belonging to section *Spirocarpos*. A lateral hilum position was present in all studied taxa except five species of section *Medicago*, which instead had both lateral and subapical hilum positions (*M. glomerata*, *M. sativa* subsp. *hemicycla*, *M. falcata*, *M. polychroa*, and *M. suffruticosa*). Hilar rim, raphe and lens were present and visible in seeds of all studied taxa and were taxonomically significant in discriminating the two varieties of *M. intertextata* (subsection *Intertextae*), which were characterized by a hilar rim and lens color lighter than the testa. In contrast, hilar rim and lens color were darker than the testa in all other taxa. Lens position relative to hilum and hilar rim distinguishes taxa of section *Medicago* with confluent lens from taxa of section *Spirocarpos* with lens adjacent to the hilum (Table 2).

Cotyledon shape, length, width and color were investigated. Three different shapes were observed: oblong, which distinguished species of section *Medicago*, and the reniform and subreniform shapes, which were present in species of section *Spirocarpos*. Cotyledon color is mainly creamy except in *M. falcata* and *M. suffruticosa* which were yellow.

Radicle shape was triangular in all studied taxa. Radicle length in relation to seed length was a significant character, discriminating species of section *Medicago*, which had a radicle longer than half the cotyledon length from species of section *Spirocarpos*, which had a radicle equal to or less than half the cotyledon length. Radicle position to cotyledons was also taxonomically significant to the identification of *M. suffruticosa* with incumbent position from all other studied taxa with accumbent radicle position except *M. falcata* which had the two states. The plumule was rudimentary in most taxa except *M. rotata* var. *rotata*, *M. truncatula* var. *truncatula*, *M. truncatula* var. *inermis*, *M. aculeata* var. *aculeata* and *M. doliata* var. *doliata* which had a moderately developed plumule.

Hypocotyl color distinguished taxa of subsections *Intertextae* and *Rotatae* (*M. intertextata* var. *intertextata*, *M. intertextata* var. *ciliaris* and *M. rotata* var. *rotata*) from species of other sections and subsections because they had a pink epicotyl rather than green hypocotyl. Cotyledonary leaf shape was oblong in the two varieties of *M. intertextata* and *M. truncatula* × *littoralis* and narrowly oblong in all other taxa. According to cotyledonary leaf area two blade classes were present: leptophyll < 25mm² and nanophyll 25-225mm². The epicotyl was green in all studied taxa except the two varieties of *M. intertextata*, which had pink epicotyl. Two types of hairs were observed: simple unicellular and glandular multicellular hairs; the indumentum varied from glabrous, sparsely to densely hairy.

Stipules were consistently lanceolate and their margins varied from entire, finely serrate, serrate, one-toothed to dentate. First foliage leaf shape varied from: (i) Oblate in *M. truncatula* × *littoralis*, *M. murex* var. *murex* and *M. aculeata* var. *aculeata*, (ii) Transversely elliptic in *M. littoralis* var. *littoralis*, *M. truncatula* var. *inermis*, *M. rigidula* var. *rigidula*, *M. rigidula* var. *agrestis*, *M. rigidula* var. *cinerascens* and *M. rigidula* var. *submitis*, to (iii) Ovate in other taxa. The area of first foliage leaves fell into two classes: leptophyll (< 25mm²)

and nanophyll (25- 225mm²). First foliage leaf apex was (i) Emarginate in *M. falcata*, *M. tornata* var. *lenticularis* and *M. rigidula* var. *cinerascens*; (ii) Retuse in *M. glomerata*, *M. sativa* subsp. *hemicycla*, *M. rigidula* var. *submitis*, *M. truncatula* var. *inermis*, *M. murex* var. *murex*, *M. tornata* var. *lenticularis* f. *aculeate*, and *M. suffruticosa*; (iii) Truncate in *M. sativa* subsp. *varia*, *M. polychroa*, and (iv) obtuse in other taxa. First foliage leaf margin was (i) Finely dentate in *M. sativa*, *M. rigidula* var. *submitis*, *M. aculeata* var. *aculeate*, and *M. tornata* var. *lenticularis* f. *aculeate*; (ii) Dentate in *M. rigidula* var. *agrestis*, *M. truncatula* var. *truncatula* and *M. truncatula* var. *inermis*; (iii) Deeply dentate in *M. rigidula* var. *rigidula* and *M. rigidula* var. *cinerascens* and (iv) Entire-shallow undulate in other taxa. First foliage leaf base may be cuneate, rounded, truncate or cordate (Table 2).

The dendrogram in Fig. 1 indicates that the studied taxa can be divided into two groups corresponding to the two traditional sections of *Medicago* and *Spirocarpos* on the basis of embryo, seed and seedling morphological characters. The first series differentiated into two subseries according to cotyledon shape and radicle length in relation to seed length. The first subseries comprised seven species belonging to section *Medicago* and was characterized by oblong cotyledons, a radicle longer than half the cotyledon length, seed lens confluent with hilum, and a glabrous first foliage leaf. The second subseries comprised two varieties of *M. tornata* (section, *Spirocarpos*) and was characterized by reniform cotyledons, and a radicle less than half the cotyledon length.

The second series included all other species of section *Spirocarpos* and differentiated into two subseries according to seed testa, hilar rim, lens color, and hair type. The first subseries comprised two varieties of *M. intertexta*, which were characterized by blackish brown testa, hilar rim, and lens lighter than testa color, the presence of glandular hairs and the absence of simple unicellular hairs. The second subseries included species characterized by yellow-dark brown testa, hilar rim and lens darker than testa color, and the presence of simple unicellular hairs. The second subseries then split off into two clusters.

The first cluster differentiated into two subclusters according to hypocotyl color, radicle length, and the presence of glandular hairs. The first subcluster contained only *M. rotata* var. *rotate*, which was characterized by a pink hypocotyl,

a radicle equal to half the cotyledon length, and the presence of glandular multicellular hairs. The second subcluster comprised three taxa, namely, *M. aculeata*, *M. doliata* var. *doliata* and *M. truncatula*, which were characterized by a green hypocotyl, a radicle less than half the cotyledon length, and the absence of glandular multicellular hairs.

The second cluster differentiated into two subclusters according to the presence of glandular multicellular hairs. The first subcluster, which comprised *M. littoralis*, *M. littoralis* var. *inermis*, *M. truncatula* var. *inermis* and *M. truncatula* var. *tricycle*, was characterized by the absence of glandular multicellular hairs. The second subcluster, which comprised four varieties of *M. rigidula* and *M. truncatula* × *littoralis*, was characterized by the presence of glandular multicellular hairs.

Discussion

The present study showed that the morphology of seeds, cotyledons, embryonic axis, seedlings, and first foliage leaf characteristics were useful tools to discriminate between the studied species and to verify the relationships between them.

The results indicated that seed and embryo morphological characters, (seed shape, cotyledon shape, color, radicle length and position and lens position) are taxonomically significant at the sectional level and can be used in delimiting taxa of section *Medicago* from that of section *Spirocarpos*. The relative length of the radicle and cotyledons in the ungerminated seeds was also a good diagnostic character for discriminating the more primitive section, *Medicago* (those relatively similar to *Trigonella*) from the more advanced section *Spirocarpos*. *Medicago* had seeds with longer radicles, typically three-quarters to as long as the length of seeds, whereas *Spirocarpos* had a relatively shorter radicle that was about half the length of the seed.

The results also indicated that seed testa color, hilar rim, lens color, and position, hypocotyl, epicotyl color and cotyledonary leaf shape characters were taxonomically significant at the subsectional level, delimiting the studied species that belong to three subsections of section *Spirocarpos* (*Intertextae*, *Rotatae*, and *Pachyspireae*) and two subsections of section *Medicago* (*Falcago* and *Suffruticosae*) (Plates 1, 2).

TABLE 2. List of 65 characters recorded comparatively for the 25 *Medicago* species and varieties listed in Table 1.

I. Seeds	
1	Shape: reniform 1/ subreniform 2/ mitaform 3/ oblong 4
2	Length (in mm)
3	Width (in mm)
4	Testa color: yellow-dark brown 1/ blackish brown 0
5	Hilum shape: circular 1/ elliptic 0
6	Hilum position: lateral 1/ sublateral-apical 0
7	Hilar rim: darker than testa 1/ lighter than testa 0
8	Lens length (in μm)
9	Lens color: darker than testa 1/ lighter than testa 0
10	Lens position relative to hilum and rim: confluent 1/ adjacent 0
11	Seed poles: rounded 1/ rounded-rounded:truncate 2/ truncate-rounded:truncate 3
II. Embryo	
12	Cotyledon shape: reniform 1/ subreniform 2/ oblong 3
13	Cotyledon length (in mm)
14	Cotyledon width (in mm)
15	Cotyledon color: creamy 1/ pale yellow 2/ yellow 3
16	Radicle length (in mm)
17	Radicle length: longer than half cotyledon length 1/ equal to half cotyledon length 2/ shorter than half cotyledon length
18	Radicle position relative to cotyledons: accumbent 1/ incumbent 2/ accumbent and incumbent 3
19	Plumule: rudimentary 1/ moderately developed 0
20	Plumule length (in μ)
III. Seedling	
21	Hypocotyl: length (in cm)
22	Hypocotyl: green 1/ pink 0
23	Hypocotyl: glabrous 1/ sparsely hairy 0
24	Cotyledonary leaf blade: oblong 1/ narrowly oblong 0
25	Cotyledonary leaf blade length (in cm)
26	Cotyledonary leaf blade width (in mm)
27	Cotyledonary leaf blade area (in mm^2): leptophyll (<25 mm^2) 1/ nanophyll (25 - 225 mm^2) 0
28	Epicotyl length (in mm)
29	Epicotyl: green 1/ pink 0
30	Epicotyl: glabrous 1/ sparsely hairy 2/ densely hairy 3
31	Simple unicellular hairs on epicotyl: present 1/ absent 0

TABLE 2. Cont.

32	Glandular hairs on epicotyl: present 1/ absent 0
33	Stipules of first foliage leaf: length (in mm)
34	Stipules of first foliage leaf: width (in mm)
35	Stipules of first foliage leaf margin: entire 1/ finely serrate 2/ serrate 3/ dentate 4/ serrate-1 toothed 5
36	Stipules of first foliage leaf, lower surface: glabrous 1/ sparsely hairy 2/ densely hairy 3
37	Stipules of first foliage leaf, simple unicellular hairs on lower surface: present 1/ absent 0
38	Stipules of first foliage leaf, glandular multicellular hairs on Lower surface: present 1/ absent 2
39	Stipules of first foliage leaf, margin: glabrous 1/ sparsely hairy 2/ densely hairy 3
40	Stipules of first foliage leaf, simple unicellular hairs on margin: present 1/ absent 0
41	Stipules of first foliage leaf, glandular multicellular hairs on margins: present 1/ absent 0
42	Petiole of first foliage leaf: length (in cm)
43	Petiole of first foliage leaf: glabrous 1/ sparsely hairy 2/ densely hairy 3
44	Petiole of first foliage leaf: simple unicellular hairs: present 1/ absent 0
45	Petiole of first foliage leaf: glandular hairs: present 1/ absent 0
46	Petiolule: glabrous 1/ sparsely hairy 2/ densely hairy-woolly 3
47	Petiolule: simple unicellular hairs: present 1/ absent 0
48	Petiolule: glandular hairs: present 1/ absent 0
49	First foliage leaf, blade shape: Ovate 1/ Oblate 2/ Transversely elliptic 3/ ovate-obcordate 4/ Ovate-oblate 5
50	First foliage leaf, length (in mm)
51	First foliage leaf, width (in mm)
52	First foliage leaf, length : width
53	First foliage leaf, area (in mm ²)
54	First foliage leaf, apex: retuse 1/ retuse-emarginate 2/ emarginated 3/ obtuse-retuse 4/ truncate-obtuse 5/ obtuse-acuminate 6
55	First foliage leaf, apex central tooth: present 1/ absent 0
56	First foliage leaf, margin: entire 1/ shallowly undulate 2/ deeply dentate 3/ dentate 4
57	First foliage leaf, upper surface: glabrous 1/ sparsely hairy 2/ densely hairy 3
58	First foliage leaf, upper surface, simple unicellular hairs: present 1/ absent 0
59	First foliage leaf, upper surface, glandular hairs: present 1/ absent 0
60	First foliage leaf, lower surface: glabrous 1/ sparsely hairy 2/ densely hairy 3
61	First foliage leaf, lower surface, simple unicellular hairs: present 1/ absent 0
62	First foliage leaf, lower surface, glandular hairs: present 1/ absent 0
63	First foliage leaf, margin: glabrous 1/ densely hairy 2/ sparsely hairy 3
64	First foliage leaf, margin: simple unicellular hairs: present 1/ absent 0
65	First foliage leaf, margin: glandular hairs: present 1/ absent 0.

TABLE 3. Data matrix comprising the comparative recording of 65 characters of the seeds, embryos and seedlings of 25 *Medicago* species and varieties

Taxa	Characters																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
<i>M. sativa</i>	2	2.35	1.25	1	1	1	1	4	1	1	1	3	2.90	1.10	1	1.93	1	1	1	1.75
<i>M. glomerata</i>	3	2.53	1.55	1	1	0	1	6	1	1	2	3	2.85	1.15	1	1.95	1	1	1	1.25
<i>M. sativa</i> subsp. <i>hemicycla</i>	3	2.25	1.43	1	1	0	1	6.5	1	1	2	3	2.75	1.17	1	1.97	1	1	1	1.25
<i>M. falcata</i>	4	1.82	1.05	1	1	0	1	5	1	1	1	3	2.17	1.07	2	1.80	1	3	1	0.75
<i>M. sativa</i> subsp. <i>varia</i>	2	2.40	1.43	1	1	1	1	4	1	1	1	3	2.95	0.95	1	1.90	1	1	1	1.75
<i>M. polychroa</i>	2	2.45	1.37	1	1	0	1	4.5	1	1	2	3	2.80	1.07	1	2.03	1	1	1	1.25
<i>M. Suffruticosa</i>	3	2.47	1.53	1	1	0	1	3.5	1	1	1	3	2.85	1.10	3	2.55	1	2	1	1.25
<i>M. intertextavar. intertextata</i>	2	4.38	2.60	0	1	1	0	9	0	1	1	2	5.40	2.20	1	2.95	2	1	1	5.5
<i>M. intertextata</i> var. <i>ciliaris</i>	2	4.52	2.82	0	0	1	0	7	0	1	1	2	5.27	3.10	1	3.00	2	1	1	8
<i>M. rotatavar. rotata</i>	2	4.53	2.67	1	0	1	1	7.5	1	0	1	2	4.93	2.23	1	2.50	2	1	0	6
<i>M. littoralis</i> var. <i>littoralis</i>	2	3.08	1.80	1	1	1	1	7.5	1	0	3	2	3.67	1.63	1	1.90	2	1	1	3
<i>M. littoralis</i> var. <i>inermis</i>	2	3.22	1.83	1	1	1	1	3.5	1	0	2	2	3.45	1.50	1	1.80	2	1	1	2.5
<i>M. rigidulavar. rigidula</i>	2	3.78	2.37	1	1	1	1	6.5	1	0	1	2	4.43	2.00	1	2.20	2	1	1	3
<i>M. rigidula</i> var. <i>agrestis</i>	2	3.85	2.15	1	0	1	1	6.5	1	0	1	2	4.17	2.03	1	2.07	2	1	1	5
<i>M. rigidula</i> var. <i>cinerascens</i>	2	3.06	1.96	1	1	1	1	7.5	1	0	1	1	4.93	2.43	1	2.60	2	1	1	5
<i>M. rigidula</i> var. <i>submitis</i>	2	5.00	2.75	1	0	1	1	7.5	1	0	1	1	5.10	2.53	1	2.30	2	1	1	4.33
<i>M. truncatula</i> var. <i>truncatula</i>	2	3.98	2.42	1	0	1	1	6.5	1	0	1	2	4.25	2.05	1	2.00	3	1	0	4
<i>M. truncatula</i> var. <i>inermis</i>	2	3.62	2.08	1	1	1	1	5.5	1	0	3	2	4.23	2.03	1	1.97	3	1	0	4
<i>M. truncatula</i> var. <i>tricycla</i>	2	3.40	1.78	1	1	1	1	4	1	0	3	2	4.13	1.93	1	1.90	2	1	1	3.33
<i>M. truncatula</i> x <i>littoralis</i>	2	3.68	2.00	1	0	1	1	6	1	0	2	2	4.27	2.00	1	1.95	2	1	1	5
<i>M. murex</i> var. <i>murex</i>	1	3.88	2.00	1	0	1	1	6	1	0	3	1	4.70	1.97	1	1.67	2	1	0	2.5
<i>M. aculeata</i> var. <i>aculeata</i>	2	4.45	2.47	1	0	1	1	9.5	1	0	1	2	5.10	2.45	1	2.35	3	1	0	7
<i>M. doliata</i> var. <i>doliata</i>	2	4.48	2.53	1	0	1	1	9	1	0	1	2	5.23	2.43	1	2.43	3	1	0	6.67
<i>M. tornata</i> var. <i>lenticularis</i>	1	3.60	2.13	1	1	1	1	6	1	0	2	1	5.17	2.03	1	2.90	2	1	1	2.75
<i>M. tornata</i> var. <i>lenticularis</i> f. <i>aculeata</i>	1	3.53	2.00	1	1	1	1	7.5	1	0	1	1	4.40	1.97	1	2.20	2	1	1	3.25

TABLE 3. Cont.

Taxa	Characters																							
	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
<i>M. sativa</i>	0.9	1	1	2	1.2	3.5	0	0.75	1	1	0	0	3.50	0.60	3	2	1	0	2	1	0	3.15	2	1
<i>M. glomerata</i>	2.3	1	1	2	1.2	3.3	0	2.33	1	1	0	0	2.73	0.47	1	1	0	0	2	1	0	1.97	2	1
<i>M. sativa</i> subsp. <i>hemicycla</i>	1.7	1	1	2	1.0	3.5	1	0.80	1	1	0	0	1.97	0.27	1	1	0	0	1	0	0	1.50	1	0
<i>M. falcata</i>	1.6	1	0	2	0.8	2.3	1	1.67	1	1	0	0	1.83	0.20	1	1	0	0	1	0	0	1.30	1	0
<i>M. sativa</i> subsp. <i>varia</i>	2.8	1	1	2	1.3	3.9	0	0.93	1	1	0	0	2.20	0.30	1	1	0	0	2	1	0	1.77	1	0
<i>M. polychroa</i>	2.8	1	1	2	1.0	3.1	1	1.67	1	1	0	0	2.00	0.33	1	1	0	0	2	1	0	1.98	1	0
<i>M. Suffruticosa</i>	2.3	1	1	2	1.2	3.2	1	0.67	1	1	0	0	1.88	0.15	1	1	0	0	2	1	0	1.55	2	1
<i>M. intertextavar. intertexta</i>	2.7	0	1	1	1.9	7.9	0	8.67	0	3	0	1	4.90	0.77	3	3	0	1	3	0	1	1.87	3	0
<i>M. intertexta</i> var. <i>ciliaris</i>	2.7	0	1	1	1.8	7.2	0	6.33	0	3	0	1	4.53	0.77	3	3	0	1	3	0	1	1.73	3	0
<i>M. rotatar. rotata</i>	4.5	0	1	2	2.3	4.8	0	5.50	1	3	1	1	2.97	0.30	3	3	1	1	3	1	1	1.43	3	1
<i>M. littoralis</i> var. <i>littoralis</i>	2.2	1	1	2	1.0	2.5	1	1.10	1	2	1	0	1.57	0.15	2	1	0	0	2	1	0	1.00	3	1
<i>M. littoralis</i> var. <i>inermis</i>	2.3	1	1	2	1.2	3.1	1	1.83	1	1	0	0	2.35	0.45	2	2	1	0	2	1	0	1.77	3	1
<i>M. rigidulavar. rigidula</i>	3.4	1	1	2	1.3	4.2	0	1.50	1	3	1	0	3.28	0.90	3	2	0	1	3	1	1	1.33	3	1
<i>M. rigidula</i> var. <i>agrestis</i>	1.9	1	1	2	1.4	4.1	0	2.17	1	1	0	0	3.30	0.55	3	3	0	1	3	1	1	1.57	3	1
<i>M. rigidula</i> var. <i>cinerascens</i>	1.9	1	1	2	1.2	3.2	0	0.60	1	2	1	0	3.20	0.40	3	2	0	1	3	1	1	1.10	3	1
<i>M. rigidula</i> var. <i>submitis</i>	2.6	1	1	2	1.8	6.6	0	3.33	1	3	1	1	4.87	0.73	4	2	1	1	2	1	1	2.10	3	1
<i>M. truncatula</i> var. <i>truncatula</i>	2.4	1	1	2	1.5	5.6	0	8.67	1	3	1	0	3.93	0.30	3	2	1	0	3	1	0	2.40	3	1
<i>M. truncatula</i> var. <i>inermis</i>	2.5	1	1	2	1.6	4.0	0	8.33	1	3	1	0	3.53	0.37	1	3	1	0	3	1	0	2.10	3	1
<i>M. truncatula</i> var. <i>tricycla</i>	1.1	1	1	2	1.3	3.7	0	1.27	1	3	1	0	2.97	0.43	1	3	1	0	3	1	0	2.17	3	1
<i>M. truncatula</i> x <i>littoralis</i>	2.0	1	1	1	1.6	6.7	0	2.33	1	1	0	0	6.17	1.10	4	3	1	1	3	1	1	1.87	3	1
<i>M. murex</i> var. <i>murex</i>	2.5	1	1	2	1.6	3.8	0	3.10	1	1	0	0	5.83	0.37	5	1	0	0	1	0	0	1.53	2	1
<i>M. aculeata</i> var. <i>aculeata</i>	2.3	1	1	2	1.8	5.8	0	1.13	1	1	0	0	5.13	0.67	3	1	0	0	2	1	0	2.43	3	1
<i>M. doliata</i> var. <i>doliata</i>	2.3	1	1	2	1.8	5.3	0	8.33	1	3	1	0	4.00	0.87	3	2	1	0	3	1	0	1.17	3	1
<i>M. tornata</i> var. <i>lenticularis</i>	4.0	1	1	2	1.9	4.8	0	5.00	1	2	1	0	2.75	0.35	5	2	1	0	2	1	0	3.25	3	1
<i>M. tornata</i> var. <i>lenticularis</i> f. <i>aculeata</i>	4.3	1	1	2	1.8	4.4	0	1.90	1	1	0	0	2.45	0.40	5	2	1	0	2	1	0	2.40	3	1

TABLE 3. Cont.

Taxa	Characters																								
	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65				
<i>M. sativa</i>	0	1	0	0	4	8.00	11.50	3	82.0	2	0	4	1	0	0	1	0	0	2	1	0				
<i>M. glomerata</i>	0	1	0	0	5	6.57	8.83	2	51.0	1	0	2	1	0	0	1	0	0	2	1	0				
<i>M. sativa</i> subsp. <i>hemicycla</i>	0	1	0	0	4	4.43	6.63	3	28.7	1	0	2	1	0	0	1	0	0	1	0	0				
<i>M. falcata</i>	0	1	0	0	4	3.33	4.97	3	18.3	3	0	2	1	0	0	1	0	0	1	0	0				
<i>M. sativa</i> subsp. <i>varia</i>	0	1	0	0	5	5.67	6.97	2	34.7	5	1	2	1	0	0	1	0	0	1	0	0				
<i>M. polychroa</i>	0	1	0	0	4	5.73	7.68	3	37.7	5	1	2	1	0	0	1	0	0	1	0	0				
<i>M. Suffruticosa</i>	0	2	1	0	1	3.60	4.65	2	14.8	1	0	1	1	0	0	1	0	0	2	1	0				
<i>M. intertextavar. intertexta</i>	1	3	0	1	5	11.20	12.97	2	119.7	4	0	1	1	0	0	2	0	1	3	0	1				
<i>M. intertexta</i> var. <i>ciliaris</i>	1	3	0	1	1	10.30	13.63	2	121.3	4	1	1	1	0	0	2	0	1	3	0	1				
<i>M. rotatar. rotata</i>	1	3	1	1	1	6.00	6.33	1	33.3	4	1	1	1	0	0	3	0	1	3	1	1				
<i>M. littoralis</i> var. <i>littoralis</i>	0	3	1	0	3	4.23	6.17	3	23.0	4	1	2	1	0	0	3	1	0	3	0	1				
<i>M. littoralis</i> var. <i>inermis</i>	0	3	1	0	4	6.77	8.67	3	49.7	4	1	2	1	0	0	3	1	0	3	0	1				
<i>M. rigidulavar.rigidula</i>	1	3	1	1	3	6.17	8.93	3	42.3	5	1	3	1	0	0	3	1	1	3	1	1				
<i>M. rigidula</i> var. <i>agrestis</i>	1	3	1	1	3	4.77	6.67	3	28.0	3	1	4	1	0	0	3	1	1	3	1	1				
<i>M. rigidula</i> var. <i>cinerascens</i>	1	3	1	1	3	4.73	6.77	3	29.3	4	1	3	3	1	0	2	1	1	3	1	1				
<i>M. rigidula</i> var. <i>submitis</i>	1	3	1	1	3	7.27	10.07	3	62.0	1	1	4	1	0	0	3	1	1	3	1	1				
<i>M. truncatula</i> var. <i>truncatula</i>	0	3	1	0	4	7.53	11.17	3	68.0	4	1	4	3	1	0	2	1	0	3	1	0				
<i>M. truncatula</i> var. <i>inermis</i>	0	3	1	0	3	5.95	8.10	3	39.3	1	1	4	2	1	0	3	1	0	3	1	0				
<i>M. truncatula</i> var. <i>tricycla</i>	0	3	1	0	1	7.17	8.97	2	50.0	6	0	2	1	0	0	3	1	0	3	1	0				
<i>M. truncatula</i> x <i>littoralis</i>	1	3	1	1	2	9.27	9.83	2	76.7	4	1	2	1	0	0	3	1	1	3	1	1				
<i>M. murex</i> var. <i>murex</i>	0	1	0	0	2	9.90	11.10	2	89.7	1	1	2	1	0	0	1	0	0	1	0	0				
<i>M. aculeata</i> var. <i>aculeata</i>	0	3	1	1	2	7.90	9.63	2	64.7	4	1	4	3	1	0	3	1	0	3	1	0				
<i>M. doliata</i> var. <i>doliata</i>	0	3	1	1	1	6.33	6.77	1	36.0	4	1	2	1	0	0	3	1	0	3	1	0				
<i>M. tornata</i> var. <i>lenticularis</i>	0	3	1	1	4	9.30	14.00	3	124.0	3	0	2	1	0	0	3	1	0	2	1	0				
<i>M. tornata</i> var. <i>lenticularis</i> f. <i>aculeata</i>	0	3	1	1	4	8.00	11.50	3	79.5	1	1	4	1	0	0	3	1	0	2	1	0				

Characters 1-65 and their states are listed in Table 2

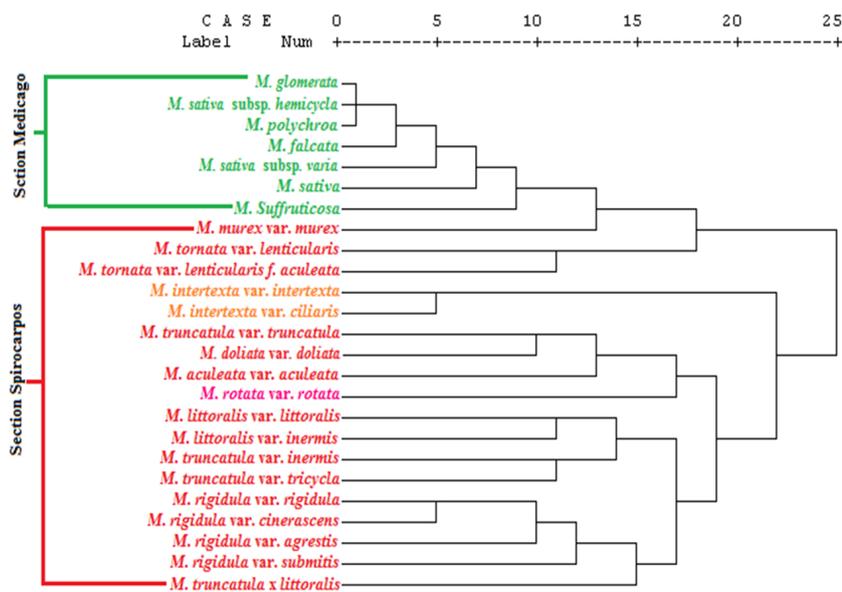


Fig. 1. Dendrogram indicating relationships between 25 *Medicago* species and varieties on the basis of 65 morphological characters of seeds, embryos, and seedlings

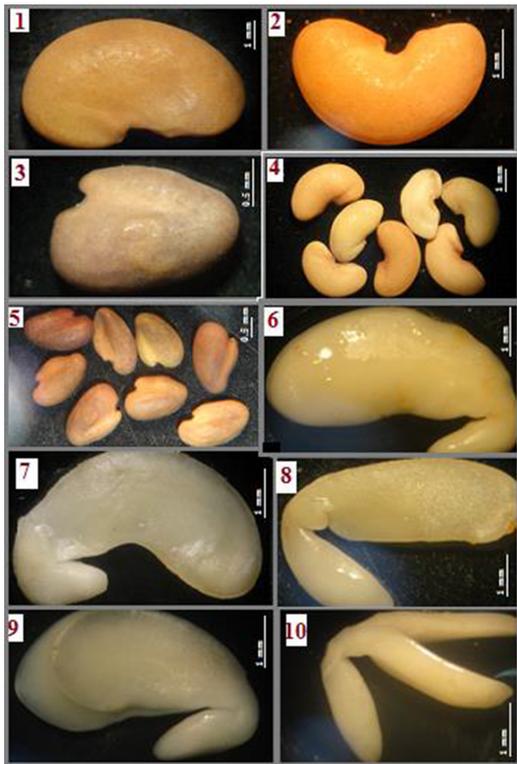


Plate 1. Seed characteristics of 25 *Medicago* species and varieties [Seed shape: (1) Subreniform, (2) Reniform, and (3) Mitaform. Hilum position: (4) Latera, and (5) Subapical. Cotyledon shape: (6) Subreniform, (7) Reniform, and (8) Oblong. Radicle position to cotyledons: (9) Accumbent and (10) Incumbent]

The seedling characteristics of the studied taxa had a taxonomic significance in delimiting the studied *Medicago* species. Variation in *Medicago* seedling morphology manifested mainly in; first foliage leaf area, shape, apex, margin, base, hairiness, hair type, and stipule margin.

The results also indicated the importance of the indumentum characters of the hypocotyl, epicotyl, and first foliage leaf. Three indumentum

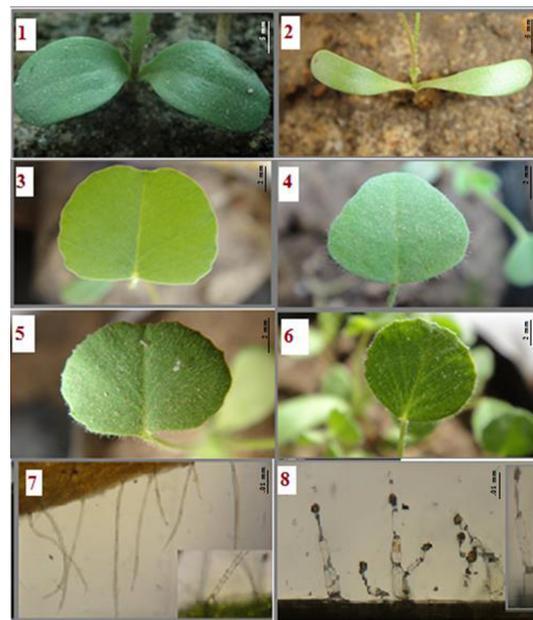


Plate 2. Seedling characteristics of 25 *Medicago* species and varieties [Cotyledonary leaf shape: (1) Oblong and (2) Narrowly oblong. Blade shape of first foliage leaf: (3) Depressed ovate, (4) Widely depressed ovate, (5) Transversely elliptic, and (6) Oblate. Hairs: (7) Simple unicellular and (8) Glandular multicellular uniseriate]

types were recorded, glabrous-sparsely hairy, densely hairy, and wooly hairy. Two different types of hairs, simple unicellular and glandular multicellular uniseriate hairs, were recorded in the studied species (Plate 2).

The present study showed that the diversity of embryo, seed and seedling morphological characters were very significant for the purpose of discrimination and identification of *Medicago* species.

Key to *Medicago* species and varieties on the basis of seed and seedling characters:

- 1. Radicle longer than half seed length.....2
 - Radicle equal or less than half seed length.....8
- 2. Radicle incumbent in position to cotyledons.....*M. suffruticosa*
 - Radicle accumbent in position to cotyledons.....3

3. First foliage leaf stipule margin serrate.....	<i>M. sativa</i>
- First foliage leaf stipule margin entire.....	4
4. First foliage leaf ovate.....	5
- First foliage leaf obovate.....	7
5. First foliage leaf apex emarginate.....	<i>M. falcata</i>
- First foliage leaf apex retuse.....	6
6. First foliage area less than 30mm ²	<i>M. sativa</i> subsp. <i>hemicycla</i>
- First foliage area more than 30mm ²	<i>M. polychroa</i>
7. Seeds metaform.....	<i>M. glomerata</i>
- Seeds subreniform.....	<i>M. sativa</i> subsp. <i>varia</i>
8. Seed testa blackish brown.....	9
- Seed testa yellow- dark brown.....	10
9. Seed hilum circular.....	<i>M. intertexta</i> var. <i>intertexta</i>
- Seed hilum elliptic.....	<i>M. intertexta</i> var. <i>ciliaris</i>
10. Hypocotyl pink in color.....	<i>M. rotata</i> var. <i>rotata</i>
- Hypocotyl green in color.....	11
11. Radicle equal to half seed length.....	12
- Radicle less than half seed length.....	17
12. Presence of glandular hairs on the first foliage leaf.....	13
- Absence of glandular hairs on the first foliage leaf.....	16
13. First foliage leaf upper surface densely hairy.....	<i>M. rigidula</i> var. <i>cinerascens</i>
- First foliage leaf upper surface glabrous.....	14
14. First foliage leaf stipule margin dentate.....	<i>M. rigidula</i> var. <i>submitis</i>
- First foliage leaf stipule margin serrate.....	15
15. First foliage leaf apex truncate - obtuse.....	<i>M. rigidula</i> var. <i>rigidula</i>
- First foliage leaf apex emarginate.....	<i>M. rigidula</i> var. <i>agrestis</i>
16. First foliage leaf transversely elliptic, its area 20- 25mm ²	<i>M. littoralis</i> var. <i>littoralis</i>

- First foliage leaf ovate, its area 40- 57mm².....*M. littoralis* var. *inermis*
17. First foliage leaf stipule lower surface densely hairy.....18
- First foliage leaf stipules lower surface glabrous-sparsely hairy.....20
18. Presence of glandular hairs on the first foliage leaf*M. truncatula* × *littoralis*
- Absence of glandular hairs on the first foliage leaf.....19
19. First foliage leaf transversely elliptic, apex retuse, margin dentate and base cuneate.....*M. truncatula* var. *inermis*
- First foliage leaf ovate, apex obtuse, margin shallow undulate and base rounded
.....*M. truncatula* var. *tricycla*
20. First foliage leaf First foliage leaf ovate, its area 40- 57mm² First foliage leaf ovate, its area 40- 57mm² ovate 21
- First foliage leaf obovate.....23
21. Upper surface of first foliage leaf, densely hairy*M. truncatula* var. *truncatula*
- Upper surface of first foliage leaf, glabrous.....22
22. First foliage leaf apex emarginate, shallow undulate margin, cordate base, upper surface sparsely hairy.
.....*M. tornata* var. *lenticularis*
- First foliage leaf apex retuse, fine dentate margin, rounded base, upper surface densely hairy.....
.....*M. tornata* var. *lenticularis* f. *aculeata*
23. First foliage leaf obovate*M. doliata* var. *doliata*
- First foliage leaf obovate.....24
24. First foliage leaf glabrous*M. murex* var. *murex*
- First foliage leaf densely hairy*M. aculeata* var. *aculeata*

Conclusion

Thus, within this limited scope of study, it is evident that the morphological features of seeds and seedlings are of value and important as that of adult vegetative and reproductive characters and as such may be used for identification and taxonomic delimitation of taxa within the genus *Medicago*.

Conflicts of interest: No conflicts of interest have been declared.

Authors contribution: Author A, conceived of the presented idea and revised the manuscript.

Author B. wrote the manuscript, analyzed and discussed the results and both contributed to the final manuscript.

Ethical approval: Not applicable

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الأهمية التصنيفية لمورفولوجيا البذور والبادرات في بعض أنواع جنس البرسيم الحجازى (الفصيلة البقولية)

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تمت دراسة مورفولوجيا البذور والبادرات لخمسة وعشرين نوعا تنتمي إلى جنس البرسيم الحجازى من العائلة البقولية من النباتات التى تم الحصول على بذورها من بنوك البذور العالمية وتم زراعتها بالكلية. هدفت الدراسة إلى اجراء مراجعة تصنيفية للأنواع قيد الدراسة من خلال دراسة الصفات الخارجية للبذور والبادرات. وذلك لاستخلاص الصفات التصنيفية وترتيبها فى جداول ولوحات وصور فوتوغرافية مجهرية دقيقة تفيد فى ايجاد العلاقات التصنيفية بين الوحدات قيد الدراسة، واستخدام تلك الخصائص كدلائل ومعايير لاستنباط المعالجات التصنيفية، وبناء مفتاح تعريفى للتمييز بين الوحدات المدروسة، بالإضافة إلى التحليل العدى بإيجاد التشابهات والاختلافات بين الوحدات التصنيفية تحت الدراسة.

ومن الدراسة تم التوصل إلى الاستنتاجات التالية:

- الأنواع قيد الدراسة تابعة لقسمين sections وهى (Medicago, Spirocarpos) وخمسه تحت قسم subsections وهى (Suffruticosae, Falcago, Rotatae, Intertextae, Pachyspireae)
- باستخدام التحليل العدى للصفات المورفولوجية وجدت العديد من الصفات الفاصلة التى يمكن أن تستخدم فى تعريف وفصل الأنواع والرتب الأقل تصنيفيا مثل varieties مثل لون البذور وشكلها، وطول الجذير بالنسبة لطول البذرة، ولون السويقة الجينية العيا وكثافة الشعيرات ونوعها، وشكل أوراق الاولية .
- باستخدام التحليل العدى للصفات المورفولوجية للبذور والبادرات تم فصل الأنواع المدروسة التابعة لجنس البرسيم الحجازى.