Heliyon 7 (2021) e06077

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

A taxonomic study of twelve wild forage species of Fabaceae

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ARTICLE INFO

Keywords:
Fabaceae
Scan electron microscope
Seed coat
Morphological characteristics

ABSTRACT

Twelve species of wild leguminosae were studied to determine similarities in the coat details of the seeds using a Scanning Electron Microscope (SEM). The numerical cluster analysis method was used to examine the morphological characteristics (98 characteristics) and to clarify the taxonomic relationship between the studied species (6 genera and 3 tribes) belonging to the Fabaceae family. The relevant wild species were: *Lotus edulis* L, *Lotus ornithopodioides* L., *Tetragonolobus purpureus* Moench, *Medicago laciniata* (L.) Mill., Gard. Dict., *M.orbicularis* (L.) Bart., *M.turbinata* (L.) All, *M.polymorpha* L., *Ononis vaginalis* Vahl, *Lathyrus aphaca* L., *Vicia sativa* L., *V. peregrine* L., and *V.tetrasperma* (L.) Schreb. The aim of this study was to produce a taxonomy reflecting the relations between these twelve forage species of Fabaceae by using the morphological and SEM features to provide a details about and clarify the relations between the examined taxa. The taxonomic histories of the Fabaceae family were reviewed. The results of the morphological description and SEM showed that it was possible to distinguish between the taxa using the cluster analysis attributes for the differences in characteristic correlation between the groups under study. This study will help researchers better grasp the classification of these species of legumes which were chosen because of the difficulty of differentiating between them, their environment benefits, their use for human consumption and pasture. The SEM is a suitable tool for this analysis, owing to the similarities exhibited by the seeds.

1. Introduction

The Legumes is among the largest families (Judd et al., 2002; Magallón et al., 2001), involving around 770 genera, and having more than 19500 species (LPWG, 2013). In economic terms, Fabaceae is only second in importance to *Poaceae* (Mabberley, 1997; Yahara et al., 2013), and is represented by 42 genera and 200 species in Libya (Jafri and El-Gadi, 1980). It has a wide global set of allocation (Stevens, 2006). The organs of Fabaceae have a high grade of differences for epidermal cell types (Cildir et al., 2012). Many studies have illustrated the use of micromorphological traits to distinguish between some taxa of Fabaceae (Albert and Sharma, 2013). The genus *Lotus,* which has about 140 species, is considered the biggest genus of the tribe *Loteae* (Kramina and Sokoloff, 2004). Studies on all species of *Lotus* in Egypt revealed that it is represented by 18 taxa (Boulos, 2009). Fifteen species represent the genus *Lotus* in Libya. From about 150 species of *Vicia,* mostly in the temperate region, 13 species were reported in Libya. Also, from about 150 species of *Lathyrus*, primarily in North America and Africa, 12 species were reported in Libya, while among the 75 species of *Ononis* in the Mediterranean region, 12 were identified in Libya. In addition, 20 species of *Medicago* were reported in Libya (Jafri and El-Gadi, 1980). The efficiency of the Fabaceae species in using atmospheric nitrogen with soil rhizobia is probably the most well-known ecological trait of the Fabaceae (Werner et al., 2014, 2015). The accepted taxonomic division of Fabaceae is into three recognized subfamilies (Wojciechowski et al., 2004; Wojciechowski, 2006). This new classification of Fabaceae is recognized widely by the Fabaceae systematics community (Azani et al., 2017). According to Stace (1984), "There is obviously no reason to believe that the developmental stages in the growth of hairs are more useful than their mature structure." In this study, 12 species of Fabaceae, subfamily Papilionaceae (Faboideae), were studied, including *Lotus edulis* L, *Lotus ornithopodioides* L., Tetragonolobus purpureus Moench, *Medicago laciniata* (L.) Mill., Gard. Dict., *M.orbicularis* (L.) Bart., *M.turbinata* (L.) All, *M.polymorpha* L., *Ononis vaginalis* Vahl, *Lathyrus aphaca* L., *Vicia sativa* L., *V. peregrine* L., and *V.tetrasperma* (L.) Schreb. These species were chosen due to the significant economic and ecological importance of these plants in Al-Jabal Al-Akhdar, eastern Libya, and due to the difficulty in distinguishing between the seeds of these species. As reported by Escaray et al. (2012) these species may be used for human consumption or animal feeds. Several studies have used SEM technology to distinguish between seeds

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https://doi.org/10.1016/j.heliyon.2021.e06077
Received 28 April 2020; Received in revised form 9 June 2020; Accepted 20 January 2021

of the legume species (Kahraman et al., 2014; Delgado et al., 2015; Ozkahraman et al., 2016). The objective of this study is to propose a classification that reflects the taxonomic relationships among twelve species of Fabaceae using a modern taxonomic method. The study is comprised of several parts, each dealing with a particular aspect of the taxonomic evidence: the morphological descriptions of the twelve species, Electron Microscope Scanning on the surface of the seeds for ease of differentiation, and numerical analysis of the aforementioned data.

2. Material and methods

In this study, twelve species belonging to the Fabaceae family representing 3 tribes and 6 genera were studied. Specimens, seeds, and plant materials were randomly collected between March to December during 2015 and 2016 from four sites in Al-Jabal Al-Akhdar, eastern Libya: 1) Al-Baida, 32° 45′ 059″N, 21° 44′ 030″E, 2) Gernada, 32° 43′ 048″N, 21° 54′ 022″E, 3) Shahat, 32° 49′ 370″N, 21° 51′ 222″E and, 4) Labraq, 32o 47′ 012″N, 21o 59' 052″E (Table 1). The species were identified according to the criteria set out by Jafri and El-Gadi (1980) and Boulos (1999). The specimens were preserved at the herbarium of the Department of Crop Science, Faculty of Agriculture, Omar Al-Mukhtar University. The present investigation aims to supply a classification that reflects the taxonomic relationships among the above species of Fabaceae and analyze the morphological characteristics (98 characteristics, Tables 2 and 3). The taxonomic evidence was collected from various sources, both morphological and micro-morphological (SEM for seed coat traits).

2.1. Morphological description

The general morphological descriptions of each species were undertaken through a study of 10 herbarium specimens and fresh plants collected from the different sites mentioned above. The fresh plants were further matched against the herbarium specimens to ensure accuracy of identification.

2.2. Seeds morphology and coat scan features

The general morphological characteristics of the plant parts were judged using an Olympus Microscope (SZX16) (Murphy, 2008). The detailed surface-scan features were examined using SEM with different dimensions of 450 mm(W) x 640 mm(D) since it was 52% more compact, 45% brighter, and 50% more energy-efficient than the current model TOKYO, Japan, 2016. The SEM-micrographs were used to facilitate the morphological descriptions of seeds. For each SEM photograph the magnification power was expressed by (X). The magnification power was up to 8000 depending on the seed-size variations to represent the clearest and the finest details of different surface sculptures. In order to identify the most important diagnostic attributes of the seeds studied, comparative tables and accumulative figures were constructed and presented in descriptive terms. Seed surface scans were used as cited by Murley (1951) and modified by Seiler (1983).

Table 1. The studied species, tribe and sites.

Tribe	Species	Site
Loteae	*Lotus edulis* L.	AlBaida, Gernada, Labraq
	Lotus ornithopodioides L.	AlBaida, Gernada, Shahat
	Tetragonolobus purpureus Moench	Gernada, Labraq, Shahat
Trifolieae	*Medicago laciniata* (L.)Mill., Gard. Dict.	AlBaida, Gernada, Labraq
	Medicago orbicularis (L.) Bart.	AlBaida, Gernada, Labraq, Shahat
	Medicago turbinata L.	AlBaida, Gernada, Labraq, Shahat
	Medicago polymorpha L.	Labraq
	Ononis vaginalis Vahl.	AlBaida, Gernada, Labraq
Vicieae	*Lathyrus aphaca* L.	Gernada, Shahat
	Vicia sativa L.	Gernada, Shahat
	Vicia peregrine L.	AlBaida, Gernada, Shahat
	Vicia tetrasperma (L.) Schreb.	AlBaida

2.3. Methods of numerical taxonomy

Numerical taxonomy, known also as phonetic, mathematical taxonomy, and multivariate morphometrics (Singh, 2010), is mainly based on the overall affinity (similarity) at any taxonomic level; i.e., species, genus, family, *etc*. In this study, the similarity or variation will be measured at the species level (represented by specimens). An equal number of specimens of each species (12 specimens) were used. The resemblance between the fundamental taxonomic units is determined in two steps: First, measuring the similarity values (or distance values) between all possible pairs of specimens under study for all of the studied characters and character states. Second, forming the similarity matrix. This matrix was analyzed using the numerical taxonomy technique supplied in the Minitab program, version 17 (Minitab, 2017).

All characters studied, including morphological, scanning, anatomical, in addition to the numerical analysis have been shown in the forms of tables, figures, plates, microphotographic pictures, and dendrograms in order to determine the similarities or dissimilarities between the studied species. The proposed keys will be established based on various posterior characters. The phenetic analysis will be based on overall affinity (resemblance). The presence of a consistent character combination defining a particular taxon is achieved by using as many characters and evidence as possible. Sokal and Sneath (1963) recommended using numerical taxonomy. All of these characters should have equal importance. The weighing of traits may take two forms and the resemblance between the classification modules can be calculated in two steps.

3. Results

The results of a morphological species description, in addition to the seed morphology and seed coat scan (micromorphology) (Table 4), seeds features of the epidermis, anticlinal walls, and outer periclinal walls (Table 5), are as follows: **Tribe 1.** *Loteae* or *Coronilleae*, Genus: *Lotus*, *Lotus edulis*. The seed outline were as follows: Reniform. Seed length: 2–3.2 mm. Width: 1.3–1.8 mm. Coat: dull. Using coat scan electron microscope at coat scan of the seed epidermal cells, power zoom 64 X (Figure 1A), 2000 X (Fig.1B) & 4000 X (Figure 1C), showed the following: raised and depressed anticlinal wall, holed and toothed outer periclinal wall, fovulariate and rugose coat scan pattern, the anticlinal walls with bigger cells 2.25–3.616 µm.

3.1. Numerical analysis

The descriptions of the 98 characters used for computation and their codes in addition to the morphological descriptions and seed coat scan features were given above. The results of the morphological description (SEM) showed that it was possible to distinguish between taxonomic taxa using cluster analysis of attributes for the difference of correlation of characteristics between the groups under study. The analysis showed that the studied species were divided into two main groups at the level of 51.02% similarity (Figure 13).

Group I: divided into

1. Those characterized at the level of 70.2% similarity. This group is further subdivided into two species: *Lathyrus aphaca* L. and *Vicia tetrasperma* (L.) Schreb.

Group II: divided into

Subgroup A at the level of 61.1% similarity includes one species *Lotus ornithopodioides* L. Also, at the level of 69.4% similarity two subgroups can be distinguished.

Table 2. Description of 98 characters and character states for morphological and numerical analysis.

1. Plant duration: 0-annual 1-Perennial
2. Habit: 0- Foetid shrub 1–2.5 m, pod 10–15 cm 1-Annual and perennial herbs or small shrubs, pod much shorter
3. The plant: 0- thorny 1- unarmed
4. Stem morphology position: 0-erect 1-ascending
5. Stems: 0- winged 1-unwinged
6. Stems: 0-smooth 1-coarse
7. Branching: 0-at lower part only 1-at lower and upper
8. Thickness: 0-thick 1-thin
9. Stem base colour: 0-green dark 1- green light
10. Node colour: 0- green dark 1- green light
11. Length of leaves: 0- From 5-15 cm 1- much short
12. Wideness: 0-wide (5 mm or more) 1-narrow (less than 5 mm)
13. Midrib: 0-prominent 1-not prominent
14. Leaves: 0- simple 1- absent
15. Leave blade margin: 0- hairy 1- glabrous
16. Leave blade vesture: 0- glabrous 1- hairy
17. Leave blade appearance: 0- shiny 1- dull
18. Sheath and Leave blade attachment point: 0- wide 1- narrow
19. Leave blade colour: 0- dark 1- pale
20. Pending of sheath and Leave blade attachment point: 0- pended 1- un-pended
21. Leaflets pedunculate: 0- present 1- absent
22. Leave sheath: 0- closed 1- opened
23. Leaves tendrils: 0- present 1- absent
24. Stipules: 0- much longer than the leaflets 1- smaller than the leaflets
25. Leaves: 0- with more than 3 leaflets 1–3 foliolate
26. Leaves: 0–3 foliolate 1- pinnate
27. Leaflets stipels: 0- present 1- absent
28. The lower surface of the leaves: 0- gland-dots pod 1–3 dispersed 1- without gland-dots pod 5–15 or more dispersed
29. Corolla: 0-withered corolla pod enclosed in the persistent calyx not exposed 1- Corolla caduceus pod exposed
30. The margins of the leaflets: 0- dentate 1- Entire
31. Leaf apices: 0 – Acute 1- Mucronate
32. Leaf bases: 0- Acute 1- Cuneate
33.small stipules1-2 mm: 0- present 1- absent
34. Leaflets 5, the basal pair stipule like, subsessile, stipules very small or 0: 0- present 1- absent
35. Shap leaflets: 0- obovat 1- linear
36. 0- Leaves paripinnate, pod 10–20 (-25) cm: 1- Leaves imparipinnate, pod 1–8.5 cm
37. Ligule: 0- present 1- absent
38. Auricles: 0- present 1- absent
39. Stipules shape: 0- cylindrical 1- compressed
40. Stipules vesture: 0- hairy 1- glabrous
41. Stipules edge: 0- membranous 1- un membranous
42. Cylindrical inflorescence: 0- present 1- absent
43. Pyramidical inflorescence: 0- present 1- absent
44. Lanceolate inflorescence: 0- present 1- absent
45. Type inflorescence: 0- panicle 1- raceme
46. Arrangement of raceme: 0- regular 1- irregular
47. Rachis vesture: 0- hairy 1- glabrous
48. No. of flowers in raceme: 0- from 1-3 1- more than 3
49. Flowers: 0- White 1- yellow, pink, red or purple
50. Flowers: 0- racemes, pod not curved 1- racemes pod mostly curved
51. Flowers, petals imbricate or valvate in bud 0- regular 1- irregular
52. 0- Flowers in many flowered racemes 1- Flowers few, in pedunculate axillary clusters
53. Flowers in terminal heads or axillary clusters, pod included in inflated hairy calyx, 1–2 seeded: 0- present 1- absent

Table 2 (*continued*)

54. 0- Corolla Petals free or wings adhering to the keel by a tooth 1- Keel very adherent rather tightly to the wing by wing spur in a keel invagination
55. 0- Pod subglobose oblong elliptic or circular 1- Pod flattened
56. 0- Pod circular 1- Subglobose or oblong-ellipsoid
57. 0- Pod indehiscent, 1–3 segmented 1- dehiscent, not segmented
58. 0- Pod 1–2 seeded 1- pod at least 3 seeded
59. Pod: 0- aerial 1- subterranean
60. Pod indehiscent, with a conspicuous crest of irregular spinose lobes: 0- present 1- absent
61. 0- Pod spirally twisted, usually spiny: 1- Pod not spirally twisted, not spiny
62. 0- Pod dehiscent, not veined: 1- Pod indehiscent, variously veined
63. 0- Pod 1 seeded with a long sword shaped beak 1- Pod without
64. Pod a loment, segments 1 seeded: 0- present 1- absent
65. 0- All or some hairs 2 armous 1- All hairs simple
66. 0- Pod falcate or coiled, smooth, of cylindrical or sausage shaped segments 1-Pod straight spiny flattened of 1–3 rounded segments
67. 0- Standard hairy on the dorsal surface 1- Standard glabrous,
68. 0- Stigma terminal, capitates 1- Stigma oblique, discoid
69. 0- Corolla Calyx 3 lobed, the lateral lobes 2 fid, flowers yellowish cream 1- Calyx not as above, flowers yellow, red, pink, purple or white
70. 0- Pod inflated 1- not inflated
71. 0- Calyx lipped deeply 2 the upper lip 2 fid, the lower 3 dented 1- Calyx not 2 lipped, the teeth subequal
72. Leaves Pedunculate: 0- Present 1- absent
73. Flower length 0- Least than 1 cm 1- More than 1 cm
74. 0- Corolla Segments of the pod ovoid-orbicular or quadrangular, spiny or spinulose, seeds reniform 1- Segments of the pod horseshoe shaped, not spiny, seeds curved or horseshoe shaped
75. Pod much inflated, membranous, indehiscent 0- Present 1- absent
76. Pod orbicular, flattened: 0- Present 1- absent
77. Stipules free, always with dark markings (glands) at the apex, pod compresse, with a 2–3 cm hooked beak 0- present 1- absent
78. 0- Pod 5–6 mm, include in the calyx, densely villous 1-Pod longer5-6mm, if included in the calyx not villous
79. Mature stage: 0- Dehiscent 1- Indehiscent
80. Seeds outline, Ellipsoid shape: 0- Present 1- absent
81. Seeds outline, Oblong shape: 0- Present 1- absent
82. Seeds length: 0- From 1 cm or more 1- less than 1 cm
83. Seeds width: 0–2 mm 1- less than 2 mm
84. Seeds surface: 0- shiny 1- dull
85. Seeds raised anticlinal wall: 0- present 1- absent
86. Seeds depressed anticlinal wall: 0- present 1- absent
87. Seeds grooved anticlinal wall: 0- present 1- absent
88. Seeds flattend outer perclinal wall: 0- present 1- absent
89. Seeds grooved outer perclinal wall: 0- present 1- absent
90. Seeds raised outer perclinal wall: 0- present 1- absent
91. Seeds holes outer perclinal wall: 0- present 1- absent
92. Seeds toothed outer perclinal wall: 0- present 1- absent
93. Seeds fovulariate surface scan pattern: 0- present 1- absent
94. Seeds rugose surface scan pattern: 0- present 1- absent
95. Seeds scalariform surface scan pattern: 0- present 1- absent
96. Seeds punctuate surface scan pattern: 0- present 1- absent
97. Seeds reticulate surface scan pattern: 0- present 1- absent
98. Seeds sulcate surface scan pattern: 0- present 1- absent

- The first subgroup at a level of similarity of 79.7%, including the species *M. laciniata* and at a level of similarity of 82.7%, including two species *M. polymorpha.* and *M. turbinata.*
- The second subgroup at a level of similarity of 69.4%, including the species *Medicago orbicularis.*

Table 3. Descriptions of numerical analysis characters, character states and codes.

Species Characters	1 L.e	2 L.o	3 T.p	4 M.l	5 M.o	6 M.t	7 M.p	8 O.v	9 L.a	10 V.s	11 V.p	12 V.t
1	0	0	0	0	0	0	0	1	0	0	0	0
2	1	1	1	1	1	1	1	1	1	1	1	1
3	1	1	1	1	1	1	1	1	1	1	1	1
4	0	1	0	1	1	1	1	0	0	0	0	0
5	1	1	1	1	1	1	1	1	0	1	1	1
6	1	1	1	1	0	0	0	1	0	0	1	1
7	0	0	0	0	0	0	0	1	0	0	0	0
8	1	1	1	1	1	1	1	0	1	1	1	1
9	0	0	0	0	0	0	0	0	0	0	0	0
10	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	0	1	0	1	1	1
12	0	0	0	0	0	0	0	0	0	1	0	1
13	0	0	0	0	0	0	0	0	1	0	0	1
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	1	0	0	1
16	1	1	1	1	0	1	1	1	0	1	1	1
17	1	1	1	1	1	1	1	1	1	1	0	1
18	1	1	1	0	0	0	0	0	1	1	1	1
19	1	1	1	1	1	1	1	1	0	1	1	1
20	1	1	1	0	0	0	0	0	1	0	0	1
21	1	1	1	0	0	0	0	1	1	1	1	1
22	0	0	0	1	1	1	1	1	0	1	1	1
23	1	1	1	1	1	1	1	1	0	0	0	0
24	1	1	1	1	1	1	1	1	0	1	1	1
25	0	1	0	1	1	1	1	1	1	1	1	1
26	0	0	0	0	0	0	0	0	1	1	1	1
27	1	1	1	1	1	1	1	1	1	0	0	1
28	1	1	1	1	1	0	0	1	1	1	1	1
29	0	0	0	0	1	0	0	1	0	1	0	1
30	1	1	1	0	0	0	0	0	1	1	1	1
31	1	1	1	1	1	1	1	1	0	1	1	0
32	0	0	1	1	0	1	0	1	0	1	0	1
33	1	1	1	1	1	1	1	1	1	0	0	1
34	0	0	0	1	1	1	1	1	1	1	1	1
35	0	0	0	0	0	0	0	0	0	0	0	1
36	1	1	1	1	1	1	1	1	1	1	1	1
37	1	1	1	1	1	1	1	1	1	1	1	1
38	0	0	1	0	0	0	0	0	1	0	1	1
39	1	1	1	0	0	0	0	0	1	0	0	0
40	0	0	0	0	0	0	0	0	1	0	0	0
41	1	1	1	1	1	0	1	1	0	1	1	1
42	0	1	0	0	0	1	1	1	1	1	0	0
43	1	0	1	1	1	0	0	1	1	1	1	1
44	1	1	0	0	1	1	1	0	0	0	0	0
45	1	1	1	1	1	1	1	1	1	1	1	1
46	0	1	0	0	0	0	0	1	0	0	0	0
47	0	0	0	0	1	0	1	0	1	0	0	0
48	0	1	0	1	0	1	0	0	0	0	0	0
49	1	1	1	1	1	1	1	1	1	1	1	1
50	0	1	0	0	0	1	1	0	0	0	0	0
51	0	0	0	0	0	0	0	0	0	0	0	0
52	1	1	1	1	1	0	1	1	1	1	1	1
53	1	1	1	1	1	0	0	1	0	0	1	1
54	1	1	0	1	0	0	1	1	0	1	1	1
55	0	1	1	0	0	0	0	1	1	1	1	1
56	1	1	1	0	0	1	0	1	1	1	1	1
57	0	0	0	0	1	0	0	0	1	0	0	1
58	1	1	1	1	1	0	0	1	1	1	1	1

(*continued on next page*)

Table 3 (*continued*)

Species Characters	1 L.e	2 L.o	3 T.p	4 M.l	5 M.o	6 M.t	7 M.p	8 O.v	9 L.a	10 V.s	11 V.p	12 V.t
59	0	0	0	0	0	0	0	0	0	0	0	0
60	0	1	0	0	1	1	0	0	1	0	0	1
61	1	1	1	0	0	0	0	1	1	1	1	1
62	1	1	1	1	0	1	1	1	0	1	1	0
63	1	1	1	1	1	1	1	1	1	1	1	1
64	1	1	1	1	1	1	1	1	1	1	0	1
65	1	1	0	1	1	1	1	0	1	1	1	1
66	1	0	0	0	0	0	1	1	0	1	1	0
67	1	1	1	1	1	1	1	1	1	1	1	1
68	0	1	0	1	0	1	1	1	1	1	0	0
69	1	1	1	1	1	1	1	1	0	1	1	1
70	0	1	0	0	0	0	1	1	1	1	1	1
71	1	0	1	1	1	1	1	1	1	0	1	1
72	0	0	0	0	0	0	0	0	1	0	1	1
73	1	0	1	0	0	0	0	1	1	1	1	0
74	0	1	0	0	1	0	0	0	1	0	0	0
75	0	0	0	0	1	1	1	0	1	0	1	1
76	1	0	1	0	0	1	0	1	0	1	1	1
77	1	0	1	1	1	1	0	0	0	0	0	0
78	1	1	1	1	1	0	1	1	1	1	1	1
79	1	1	1	1	0	1	1	1	0	1	1	0
80	0	0	0	0	1	0	0	0	0	0	1	1
81	0	1	1	1	0	0	0	1	1	0	1	1
82	1	1	1	1	1	1	1	1	1	1	1	1
83	0	1	0	1	0	0	1	1	0	1	0	0
84	1	0	0	1	1	0	0	1	1	0	0	0
85	0	0	0	0	0	0	0	0	0	0	0	1
86	0	1	0	0	1	0	0	0	0	0	0	0
87	0	1	0	0	0	0	0	0	0	0	0	0
88	0	0	0	0	0	0	0	1	0	0	0	0
89	1	1	1	1	0	1	1	0	1	0	0	1
90	1	0	1	1	0	1	1	0	1	0	1	1
91	0	1	0	0	0	1	0	0	0	0	0	1
92	0	0	1	1	0	1	1	0	1	1	1	1
93	0	1	1	0	0	0	0	0	0	0	0	0
94	0	1	0	1	0	1	0	0	0	1	1	1
95	1	0	1	1	1	1	1	1	0	1	1	0
96	0	1	0	0	0	0	0	1	1	0	0	0
97	0	0	0	0	0	0	0	0	0	0	0	0
98	0	0	0	1	0	1	1	0	0	1	0	0

Key of species

1- *Lotus edulis* L, 2- *Lotus ornithopodioides* L., 3-Tetragonolobus purpureus Moench, 4-*Medicago laciniata* (L.) Mill., Gard. Dict., 5-*M.orbicularis* (L.) Bart., 6-*M.turbinata* (L.) All, 7-*M.polymorpha* L., 8-*Ononis vaginalis* Vahl, 9-*Lathyrus aphaca* L., 10- *Vicia sativa* L., 11-*V. peregrine* L., 12-*V.tetrasperma* (L.) Schreb.

Subgroup B at the level of 70.2% similarity includes the following:

- *Ononis vaginalis*, at the level of similarity of 72.1% within this sub-sub group are two species at the level of similarity of 88.7% *L. edulis.* and Tetragonolobus purpureus.
- At a level of similarity of 81.4% are two species *V. peregrina* and *V. sativa.*

Lathyrus aphaca is closer in its characteristics to all species studied for *V. tetrasperma*. Species *Ononis vaginalis* is closer in its characteristics to the studied species Tetragonolobus purpureus and *L. edulis*. Also, *V. peregrina*. and *V. sativa*.

Keys that already considered, based on the analysis technique SEM, *M. turbinata*, and *M. laciniata* had the most similarities species, 93.3% plus *M. polymorpha* at the level of similarity 87.3% (Figure 14).

Group I: includes four species at the level of 41.3% similarity divided into: *Ononis vaginalis*, *M. orbicularis* and *L. ornithopodioides*.

Group II: can be divided into the following at the level of 47.5% similarity:

A. at the level of 62.6% similarity, includes four species *Lathyrus aphaca*, *L. edulis*, Tetragonolobus purpureus, *L. ornithopodioides*.

B. at the level of 75.9% similarity: *M. laciniata*, *M. turbinata*, *M. polymorpha*, *V. peregrine*, and *V. sativa*.

3.2. Key based on the general morphological characters

A. Pod ovoid-orbicular, terete, seeds reniform, features of epidermis fovulariate

1- Pod spirally coiled, several-seeded, racemes shorter than the leaves, deflexed in fruit; flowers bright yellow; coil surface of the pod

Table 4. Morphological description of the seeds of the studied species.

No.	Species	Shape	Colour	Length mm	Width mm	L x W mm^2	Graded
1	*Lotus edulis* L.	Reniform	Dark brown	2–3.2	1.3–1.8	4.03	S
2	*Lotus ornithopodioides* L.,	Orbicular	dark brown	1.2–2	1–1.8	2.24	S
3	Tetragonolobus purpureus Moench	Orbicular	Brown	2.5–4	2–3.3	8.613	L
4	*Medicago laciniata* (L.)Mill., Gard. Dict.	Reniform, oblong-ovoid	Yellowish-brown	2.2–3	1–1.4	3.12	S
5	*M.orbicularis* (L.) Bart.,	Trigonous, compressed	Yellow to reddish-brown.	1.6.-2.3	1–1.4	2.34	S
6	*M.turbinata* (L.) All.,	Reniform	pale-brown	2–3.4	1.1–1.7	3.78	S
7	*M.polymorpha L.*	Reniform, ellipsoid-oblong	pale-brown	1.7–2.5	1–1.6	2.73	S
8	*Ononis vaginalis*Vahl.	Ellipsoid	yellow-brown	1–2.2	1.1–1.5	2.08	S
9	*Lathyrus aphaca*L.	Oblong	Dark brown	2–3	2–4	7.5	L
10	*Vicia sativa* L.	Orbicular	Yellowish-brown	2.4–3.6	1.4–2.1	5.25	L
11	*V. peregrina*L.	Spherical	Dark brown	3–4	2–2.5	7.875	L
12	*V.tetrasperma* (L.) Schreb.	Orbicular	brown-black	1.3–2	1–1.4	1.98	S

Table 5. Micro-morphological description of the seeds of the studied species.

No.	Character Species	Features of epidermis	Anticlinal walls		Outer periclinal walls
			Level	Coat	
1	*Lotus edulis* L.	Fovulariate, Rugose	Raised, Depressed,	Subglabrous	Holed Toothed
2	*Lotus ornithopodioides* L.,	Sulcate, Scalariform	raised, straight	Glabrous	Ribbed, Flattend
3	Tetragonolobus purpureus Moench.	Rugose, Prolate	Raised, straight	Glabrous	Tabular
4	*Medicago laciniata* (L.)Mill.	Fovulariate, Punctuate,	Raised, Depressed,	Glabrous	Flattend, Holed
5	*M.orbicularis* (L.) Bart.,	Fovulariate, Rugose	Raised, Grooved	Glabrous	Flattend, Grooved
6	*M.turbinata* (L.) All.,	Fovulariate, Punctuate	Raised, Depressed	Glabrous	Flattend
7	*M.polymorpha L.*	Fovulariate, Rugose	Raised, Depressed	Glabrous	Flattend, holed
8	*Ononis vaginalis*Vahl.	Rugose, Reticulate	Raised, Depressed	Subglabrous	GroovedRaised
9	*Lathyrus aphaca*L.	Fovulariate, Rugose	Raised, Depressed	Glabrous	Flattend, Holed
10	*Vicia sativa* L.	Fovulariate, Punctuate	Raised, Depressed	Glabrous	Grooved, Raised
11	*V. peregrina*L.	Fovulariate, Punctuate	Raised, Depressed	Glabrous	Flattend, Grooved
12	*V. tetrasperma* (L.) Schreb.	Fovulariate, Scalariform	DepressedGrooved	Glabrous	Flattend

distinctly reticulate; spines horizontal, thick, or reduced to tubercles *M. polymorpha*

2- Spines much shorter than the diameter of the pod, not hooked at the tip *M. turbinate*

3- Stipules coarsely toothed or laciniate; racemes 1 to 2 (-3) flowered; coil surface of the pod with 6-16 prominent S-shaped radial veins, some of them branched, Leaflets pilose or pubescent *M. laciniata*

4- Pod reniform or ovoid, 1–2 seeded, Pod 1.2–1.5 (-2) cm diam., unarmed, seeds tuberculate *M. orbicularis*

B. Pod flattened, oblong, seeds terete

B1. Foliolate 3, corolla yellow, Features of epidermis fovulariate rugose

1- Pod inflated, 5–7 mm diam., with a deep longitudinal ventral suture *L. edulis*

+ Pod inflated, 5–8 mm diam., the margins bordered by 4 conspicuous undulate wings Tetragonolobus purpureus

2- Pod flattened, strongly torulose, Pod terete or slightly compressed *L. ornithopodioides*

+ Pod flattened, Leaves sessile or subsessile; stipules sheathing *Ononis vaginalis*

B2. Leaves pinnate, corolla purple, Features of epidermis reticulate and fovulariate

1- Leaflets 0.25–1.5 cm broad, oblong, obovate, obcordate or elliptic; calyx-teeth 0.3–1.2 cm, *V. sativa*

+ Leaflets 1–2.5 mm broad, narrowly linear, calyx teeth 1.5–2 mm, corolla blue, violet, purple or white *V. peregrina*

2- Leaflets 3–6 pairs; peduncle ± equaling the leaf; pod 0.8–1.2 cm mostly 3–4 seeds, Tendrils well-developed; pod not constricted between the seeds *V. tetrasperma*

+ Leaves reduced to simple filiform tendrils; stipules large, leaf, leaf-Like, corolla yellow to yellowish *Lathyrus aphaca*

4. Discussion

Besides the micro-morphological details, the SEM matrix produces a better resolution of Fabaceae phylogeny. *M. turbinata* and *M. laciniata* were the most similar species based on the analysis technique (93.3%). The taxonomy of *Lotus* is intricate and requires an inclusive taxonomic audit of the genus (Degtjareva et al., 2011). Also, Zareh et al. (2017) stated that the anticlinal wall cells varied among the studied *Lotus edulis* L, *Lotus ornithopodioides* L., Tetragonolobus purpureus Moench, *Medicago laciniata* (L.) Mill., Gard. Dict., *M.orbicularis* (L.) Bart., *M.turbinata* (L.) All, *M.polymorpha* L., *Ononis vaginalis* Vahl, *Lathyrus aphaca* L., *Vicia sativa* L., *V. peregrine* L., and *V. tetrasperma* (L.) Schreb. The species *L. ornithopodioides* was morphologically close to all species studied of the genus *Medicago* at the a level of similarity 69.4%. Loi et al. (2017) found that the distinction between the *Lotus* species is important, where *L. ornithopodioides* germplasm was used for the development of brand-new annual self-reseeding pulse resource for Mediterranean

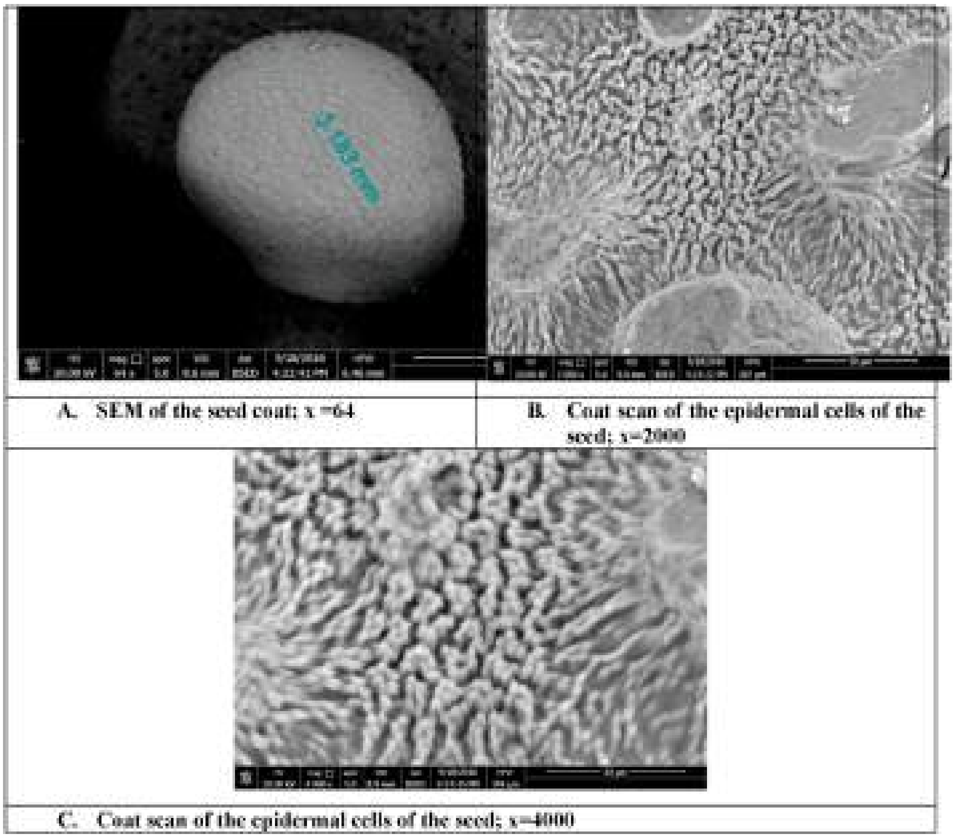


Figure 1. Seed morphology and coat scan of *Lotus edulis* L. A. SEM of the seed coat; x =64. B. Coat scan of the epidermal cells of the seed; x = 2000. C. Coat scan of the epidermal cells of the seed; x = 4000. **Tribe 1.** *Loteae* or *Coronilleae*, Genus:*Lotus*, *Lotus ornithopodioides*. The seed outline were as follows: Orbicular. Seed length: 1.2–2 mm. Width: 1–1.8 mm.Coat:glabrous. Texture: shiny. Using a coat-scan electron microscope at coat scan of the seed epidermal cells, power zoom 110 X (Figure 2A) & 8000 X (Figure 2B) showed the following: raised and flattend anticlinal wall, raised and toothed outer periclinal wall, scalariform and reticulate coat scan pattern, and glabrous anticlinal wall texture.

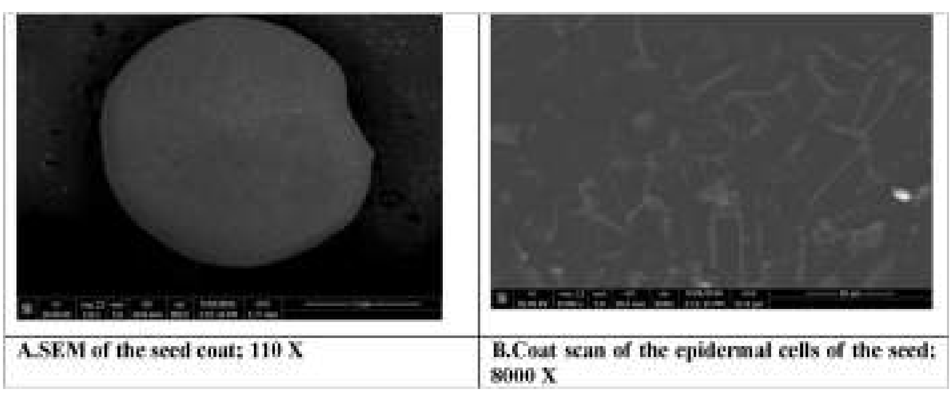


Figure 2. Seed Morphology and Coat Scan of *Lotus ornithopodioides* L. A.SEM of the seed coat; 110 X. B.Coat scan of the epidermal cells of the seed; 8000 X. **Tribe 1.** *Loteae* or *Coronilleae*, Genus: *Tetragonolobus,* Tetragonolobus purpureus Moench. Seed length: 2.5–4 mm. Width: 2–3.3 mm. Coat: glabrous. Texture: dull. Using a coat scan electron microscope at coat scan of the seed epidermal cells, power zoom 59 X (Figure 3A) & 8000X (Figure 3B), showed the following: raised and depressed anticlinal walls, holed outer periclinal wall, rugose and punctuate coat scan patterns, and glabrous anticlinal wall texture with bigger cells 2.196–2.914 μm wide.

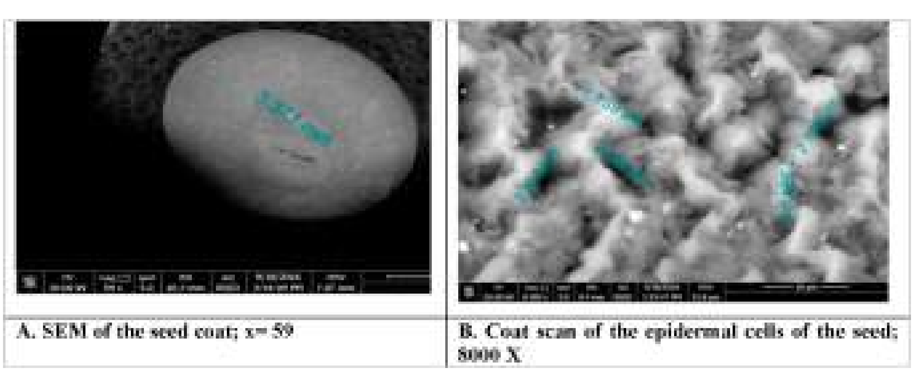
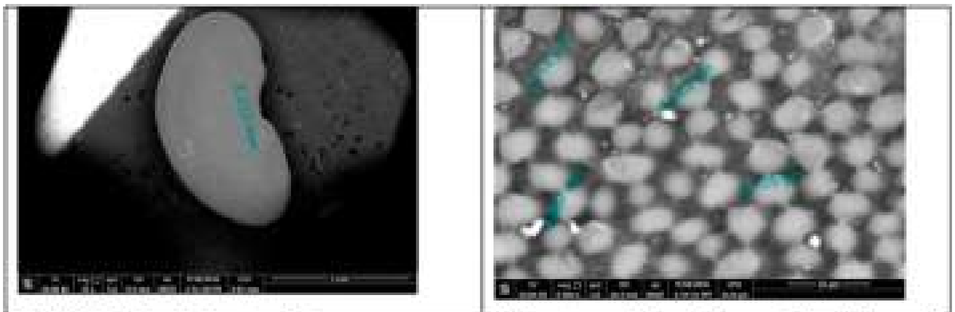
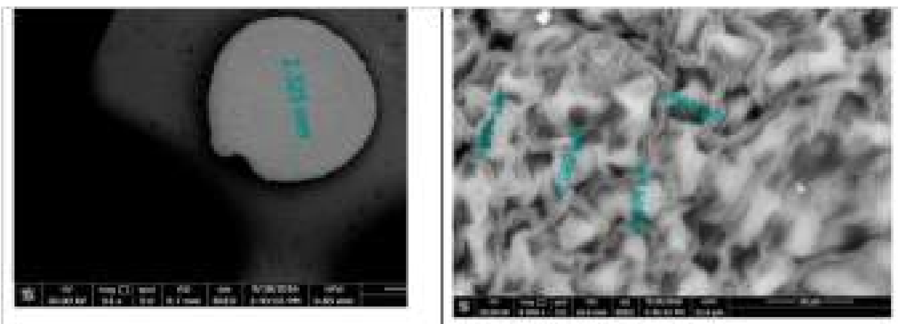


Figure 3. Seed morphology and coat scan of *Tetragonolobus purpureus* Moench. A. SEM of the seed coat; x= 59. B. Coat scan of the epidermal cells of the seed; 8000 X. **Tribe 2.** *Trifolieae*, Genus: *Medicago*, *Medicago laciniata.* Seeds outline were as follows: length: 2.2–3 mm. Width: 1–1.4 mm. Coat: glabrous. Texture: dull. Using a coat scan electron microscope at coat scan of the seed epidermal cells, power zoom 70 X (Figure 4A) & 8000 X (Figure 4B), showed the following: raised and depressed anticlinal walls, holed outer periclinal wall, fovulariate and punctuate coat scan pattern, and glabrous anticlinal wall coat with bigger cells 4.714–5.022 μm wide.



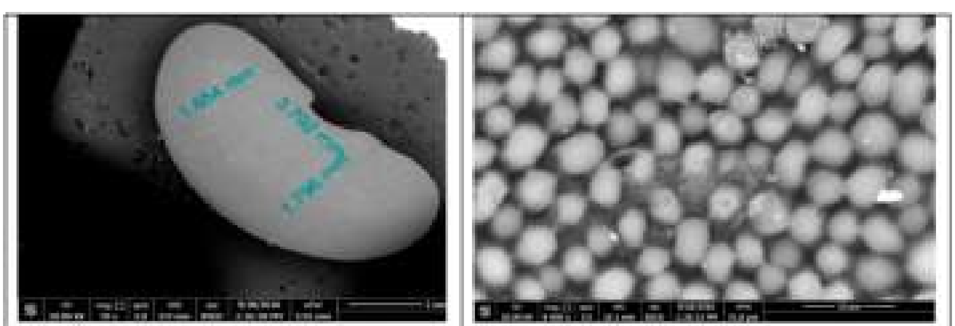
A. SEM of the seed coat; x =70 | B. Coat scan of the epidermal cells of the seed; 8000 X

Figure 4. Seed morphology and coat scan of *Medicago laciniata* (L.)Mill. A. SEM of the seed coat; x =70. B. Coat scan of the epidermal cells of the seed; 8000 X. **Tribe** 2. *Trifolieae*, Genus: *Medicago*, *Medicago orbicularis.* Seeds outline were as follows: length: 1.6.-2.3mm. Width: 1–1.4mm. Coat: glabrous. Texture: dull. Using a coat scan electron microscope at coat scan of the seed epidermal cells, power zoom 61 ((Figure 5A) & 8000X (Figure 5B), showed the following: raised and grooved anticlinal walls, flattened and grooved outer periclinal walls, fovulariate and rugose coat scan pattern, and glabrous anticlinal wall texture with bigger cells 4.831–5.095 μm wide.



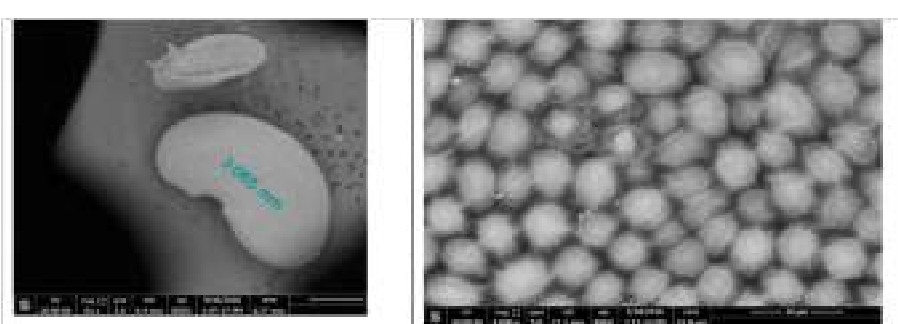
A. SEM of the seed coat; 61 X | B. Coat scan of the epidermal cells of the seed; 8000 X

Figure 5. Seed morphology and coat scan of *Medicago orbicularis* (L.) Bart. A. SEM of the seed coat; 61 X. B. Coat scan of the epidermal cells of the seed; 8000 X. **Tribe** 2. *Trifolieae*, Genus: *Medicago, Medicago turbinata*. Seed Morphology and Coat Scanoutlins were as follows: Seed length: 2–3.4 mm. Width: 1.1–1.7 mm. Coat:glabrous. Texture: shiny. Using a coat scan electron microscope at coat scan of the seed epidermal cells, power zoom 70 X (Figure 6A) & 8000 X (Figure 6B), showed the following: raised and depressed anticlinal walls, flattend outer periclinal walls, fovulariate and punctuate coat scan pattern, and glabrous anticlinal wall coat.



A.SEM of the seed coat; 70 X | B. Coat scan of the epidermal cells of the seed; 8000 X

Figure 6. Seed morphology and coat scan of *Medicago turbinata* (L.) All., A.SEM of the seed coat; 70 X. B. Coat scan of the epidermal cells of the seed; 8000 X. **Tribe 2.** *Trifolieae*, Genus: *Medicago, Medicago polymorpha*. Seeds outline were as follows: Length: 2.5 mm.Width: 1.6 mm. Coat: glabrous. Texture: shiny. Using a coat scan electron microscope at coat scan of the seed epidermal cells, power zoom 61 ((Figure 7A) & 8000 X (Figure 7B), showed the following: raised and grooved anticlinal walls, flattend and holed outer periclinal walls, fovulariate and rugose coat scan pattern, and glabrous anticlinal wall texture.



A.SEM of the seed coat; 61 X | B. Coat scan of the epidermal cells of the seed; 8000 X

Figure 7. Seed morphology and coat scan of *Medicago polymorpha*L. A.SEM of the seed coat; 61 X. B. Coat scan of the epidermal cells of the seed; 8000 X. **Tribe** 2. *Trifolieae*, Genus: *Ononis, Ononis vaginalis*. Seed Morphology and Coat Scanoutlins were as follows: Seed length: 1–2.2mm. Diameter: 1.1–1.5 mm. Coat: hairy. Texture: dull. Using a coat scan electron microscope at coat scan of the seed epidermal cells, power zoom 130 X (Figure 8A), 2000 X (Fig.8B), 4000 X (Fig.8C) & 8000 X (Figure 8D), showed the following: raised and depressed anticlinal walls, grooved and raised outer periclinal walls, and fovulariate and rugose coat scan pattern.

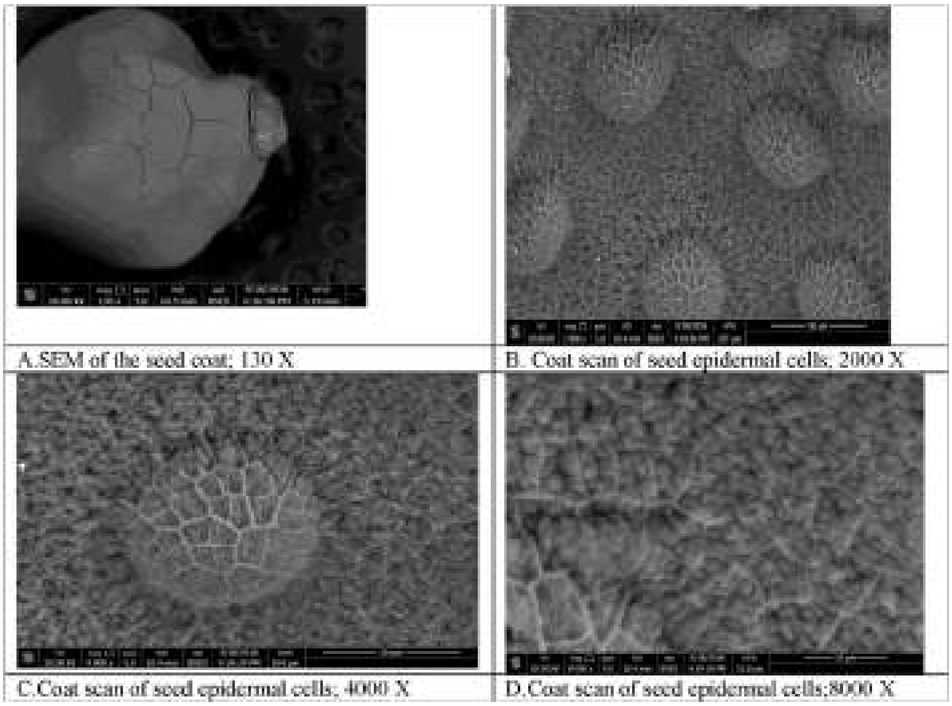


Figure 8. Seed morphology and coat scan of *Ononis vaginalis*Vahl. A.SEM of the seed coat; 130 X. B. Coat scan of seed epidermal cells; 2000 X. C.Coat scan of seed epidermal cells; 4000 X. D.Coat scan of seed epidermal cells; 8000 X. **Tribe 3.** *Vicieae*, Genus:*Lathyrus, Lathyrus aphaca*. Seed Morphology and Coat Scanoutlins were as follows: Seed length: 2–4mm.Diameter:2–3 mm.Coat: dark brown, smooth, and glabrous. Texture: dull. Using a coat scan electron microscope at coat scan of the seed epidermal cells, power zoom 75 X (Figure 9A) & 8000 X (Figure 9B), showed the following: raised and depressed anticlinal walls, flattend and holed outer periclinal walls, glabrous anticlinal wall texture, fovulariate and rugose coat scan pattern.

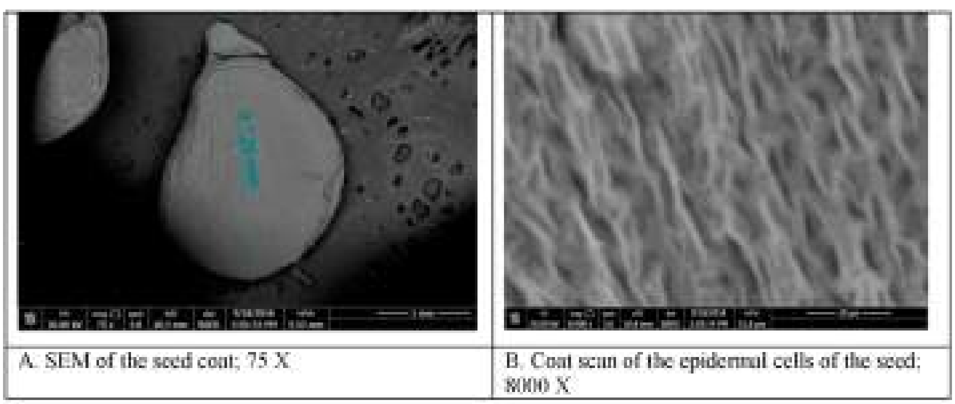


Figure 9. Seed morphology and coat scan of *Lathyrus aphaca*L. A. SEM of the seed coat; 75 X. B. Coat scan of the epidermal cells of the seed; 8000 X. **Tribe 3**. *Vicieae*, Genus: *Vicia, Vicia sativa*. Seeds outlines were as follows: Seed length: 2.4–3.6 mm. Diameter: 1.4–2.1 mm. Coat: Yellowish-brown. Texture: shiny. Using a coat scan electron microscope at coat scan of the seed epidermal cells, power zoom 59 X (Figure 10A) & 16000 X (Figure 10B), showed the following: raised, depressed, and grooved anticlinal walls, flattend and grooved outer periclinal walls, fovulariate and punctuate coat scan pattern, and sub-globose anticlinal wall texture.

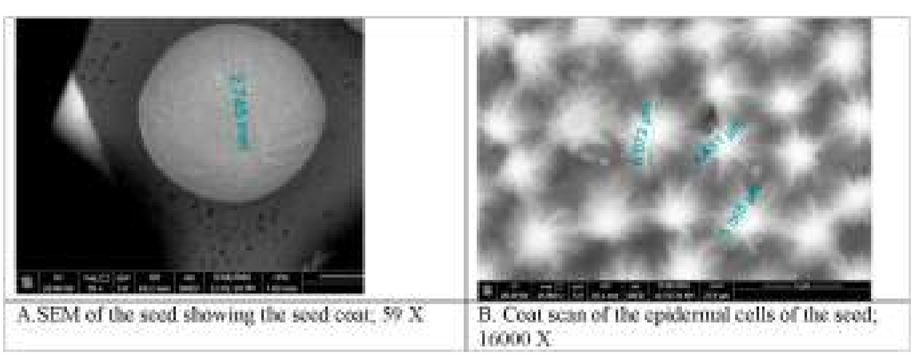


Figure 10. Seed morphology and coat scan of *Vicia sativa* L. A.SEM of the seed showing the seed coat; 59 X. B. Coat scan of the epidermal cells of the seed; 16000 X. **Tribe 3**. *Vicieae*, Genus: *Vicia, Vicia peregrina*. Seed length: 3–4mm. Diameter: 2–2.5 mm. Coat: dark brown, and glabrous. Texture: shiny. Using a coat scan electron microscope at coat scan of the seed epidermal cells, power zoom 75 X (Figure 11A) and 8000 X (Figure 11B), showed the following: raised and depressed anticlinal walls, flattend and grooved outer periclinal walls, fovulariate and punctuate coat scan pattern, and sub-globose anticlinal wall texture with bigger cells 3.691–4.464 μm wide. The pattern of seed sculpture alone does not provide sufficient details for distinguishing parts of this genus.

cultivation systems for both forage yield and forage rotation. As stated above, Avalos and Salinas (2003) highlighted the scarcity of research on scanning electron microscope analysis for the species *L.edulis*. Trichomes and features of the epidermal cells are used to identify a specific taxon. Previous studies have conclusions that Tetragonolobus purpureus cannot be genetically differentiated from *Lotus* (Sokoloff, 2006). The distinction between Tetragonolobus purpureus and *L.edulis* was based on pod inflated, 5–7 mm diam., with a deep longitudinal ventral suture of

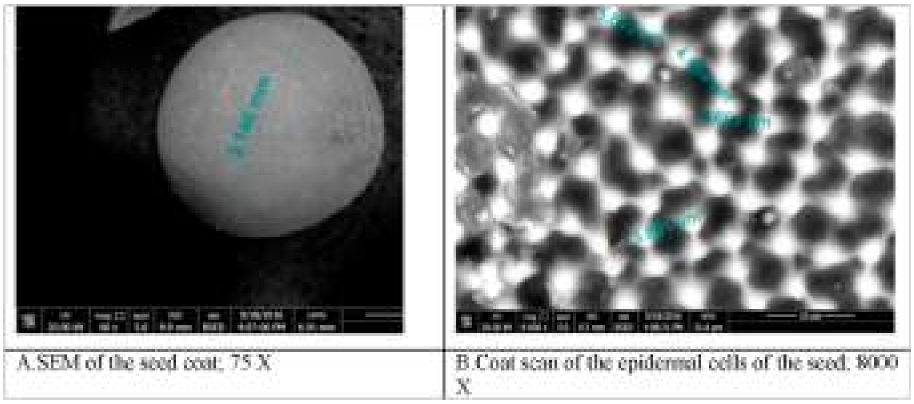


Figure 11. Seed morphology and coat scan of *Vicia peregrina*L. A.SEM of the seed coat; 75 X. B.Coat scan of the epidermal cells of the seed; 8000 X. **Tribe 3.** *Vicieae*, Genus: *Vicia*, *Vicia tetrasperma*. Seeds outlines were as follows: Seed length: 1.3–2mm. Diameter:1–1.4 mm. Coat: brown-black, and smooth. Hilum: short, ovate, and brown. Texture: shiny. Using a coat scan electron microscope at coat scan of the seed epidermal cells, power zoom 140 X (Figure 12A) and 8000 X (Figure 12B), showed the following: depressed and grooved anticlinal walls, flattend outer periclinal walls, fovularíate and scalariform coat scan pattern, and glabrous anticlinal wall coat.

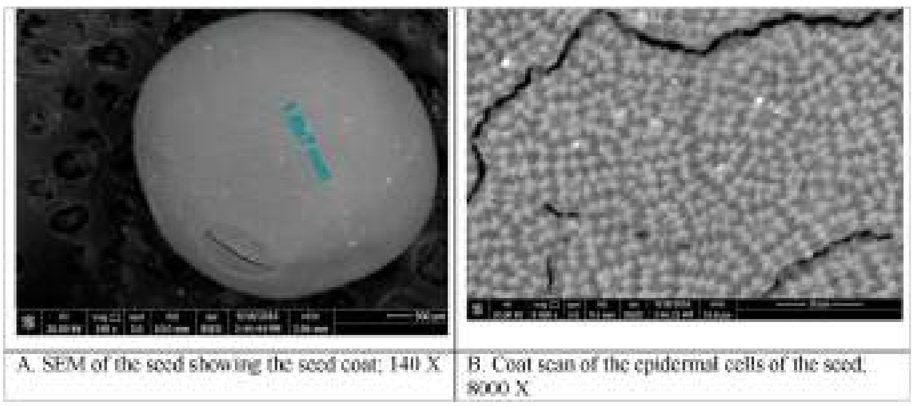


Figure 12. Seed morphology and coat scan of *Vicia tetrasperma* (L.) Schreb. A. SEM of the seed showing the seed coat; 140 X. B. Coat scan of the epidermal cells of the seed; 8000 X.

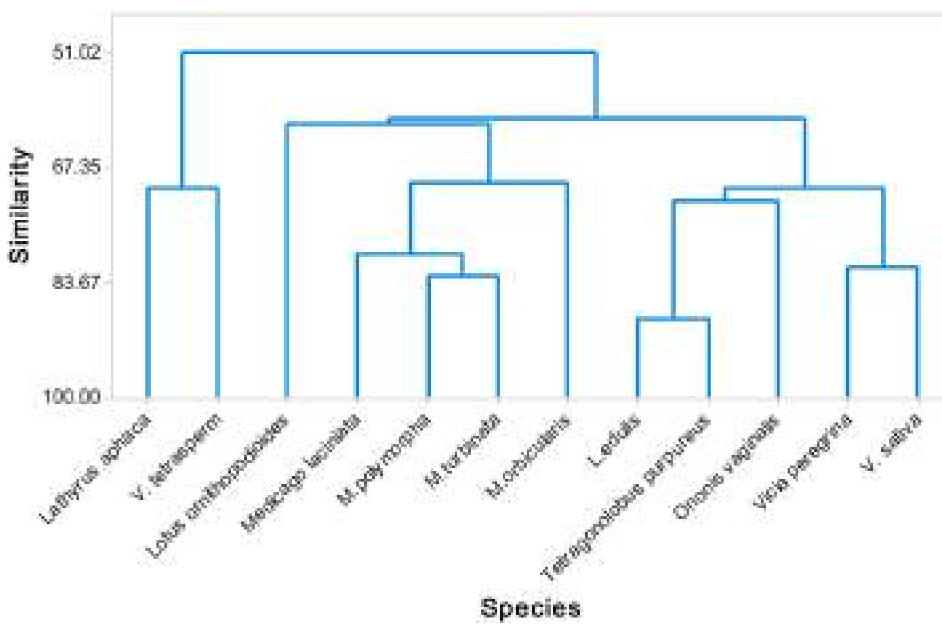


Figure 13. Cluster dendrogram of the 12 taxonomic modules based on a similarity matrix using single linkage analysis technique to all studied characters (Minitab software).

L. edulis, whereas, Tetragonolobus purpureus the pod inflated, 5–8 mm diam., and the margins were bordered by 4 conspicuous undulate wings.

Arambarri (2000) and Zareh et al. (2017) stated that the seed coat sculpture exhibited by the genus *Lotus* was reticulate, rugose, verrucate, and sulcate. These characteristics can serve as prognosis characters of the *Lotus* species-genus. The seed outline of Tetragonolobus purpureus distinguishes the species is: orbicular; coat features: rugose and prolate, dimensions: 3mm. However, Patane and Gresta (2006) and Dudeja et al.

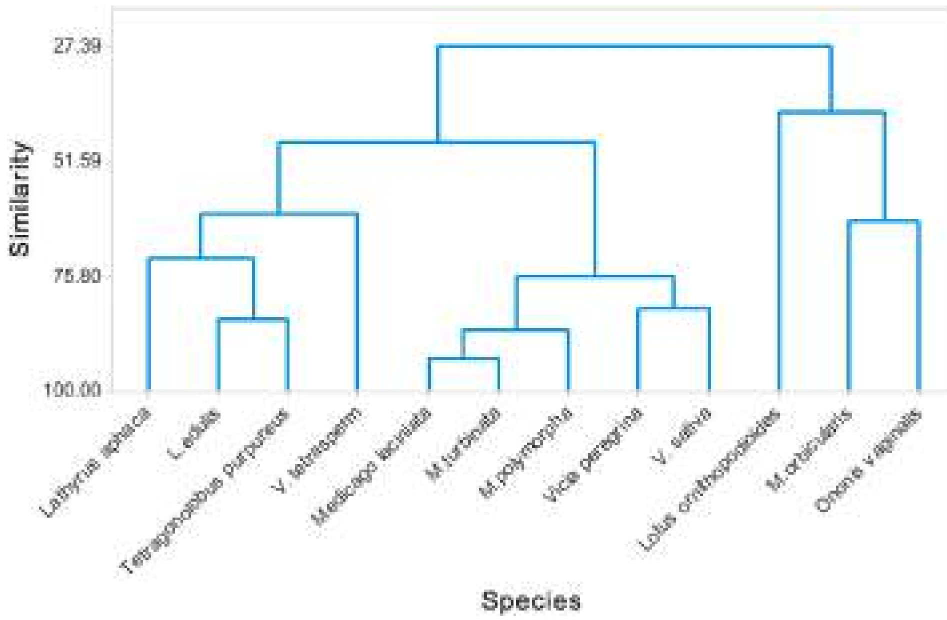


Figure 14. Cluster dendrogram of the 12 taxonomic modules based on a similarity matrix using a single linkage analysis technique to SEM studied characters (Minitab software).

(2011) found that observations of the *M.orbiculairis* by scanning electron microscope revealed the presence of a thicker layer of integument palisades cells. *M. Laciniata* features stipule coarsely toothed or laciniate; racemes (-3) flowered; Leaflets pilose or pubescent. The species *M. polymorpha*. and *M. turbinata* were too similar in its characteristics to species of the genus *Medicago* at a level of similarity of 82.7%.

The seed shape of *M. polymorpha* was reniform and ellipsoid-oblong. Meanwhile, the anticlinal wall texture of the seeds were raised and depressed. We agree with Zeng et al. (2005) that the anticlinal wall texture of the seeds of *M.polymorpha* was as "Circle Valley". The anticlinal walls of *Ononis vaginalis* are raised, depressed. Our results are consistent with Fayed et al. (2019) which showed that *Ononis vaginalis* have raised and straight anticlinal walls, and convex outer percilinal wall. Chernoff et al. (2013) showed that *Lathyrus* seems to be the most diverse in seed coat patterns compared to other seed-character groups. The differences between *Lathyrus aphaca* and *V. tetrasperma* were based on leaves, and reduced to simple filiform tendrils; stipules large, leaf, leaf- Like, corolla yellow to yellowish of *Lathyrus aphaca*, while, *V. tetrasperma* was leaflets 3–6 pairs; peduncle ± equaling the leaf; pod 0.8–1.2 cm mostly 3–4 seeds, Tendrils well-developed; pod not constricted between the seeds.

On the other hand, Büyükkartal et al. (2013) found that seed size varied significantly among the examined taxa (*Vicia*), the level of periclinal wall cells was sharply papillose in *V. peregrina*, the boundaries of anticlinal wall cells, the supporters of epidermal cells boundaries are not usually well developed, slightly immersed, slightly undulated, stellate cells in *V.peregrina*; the seed colors of *V.peregrine* are red-brown; and the level of periclinal wall cells was sharply papillose in *V.peregrina*. Generally, species of *Vicia* have a common pattern of seed sculpture which may be species-specific in some cases. The two species *V. peregrina* and *V. sativa* at a level of similarity of 81.4% where *V. peregrina* includes leaflets 1–2.5 mm broad, narrowly linear, calyx teeth 1.5–2 mm, corolla blue, violet, purple or white, whereas, *V. sativa* includes leaflets 0.25–1.5 cm broad, oblong, obovate, obcordate or elliptic and calyx-teeth 0.3–1.2 cm, as mentioned in the above description of surface morphology of the pattern *V. sativa*. As a result, seed micromorphology, demonstrated variability and taxonomic importance, with few exceptions, as it was important to distinguish taxa at the level of the species (Rashid et al., 2018). Seed colors for *V.sativa* were yellowish-brown and 5.25 mm in diameter. Whereas, Abdel Khalik and Al-Gohary (2013) found that the largest seed sizes of globular *V.sativa* to be *L. subsp*. *Sativa* seeds have a diameter of 4–6 mm and seed colors were brown-black. They also found that the smallest measures of 1.5–2 mm in *V. tetrasperma*, measuring 2.4–4 mm diameter, and the colors of the seed were yellowish.

5. Conclusions

Using SEM to examine the seed coats of species is a practical way to confirm the similarity between species. It is clear that the shape of the decorative coat of the seed is one of the most important and distinctive taxonomic characteristics of the separation of classification modules studied at species level which can be used to distinguish between these species. The two most close species in seed coat according to the SEM results were *M.laciniata* and *M.turbinate*. Also, *M.polymorpha* was relatively similar to other species in its genus. The morphological description results indicated that the two most similar species were *L.edulis* and Tetragonolobus purpureus. The species *L.ornithopodioides* was closer in its characteristics to all species studied belonging to the genus *Medicago*. The species *Lathyrus aphaca* was also closer, based on its characteristics, to all studied features of the species *V.tetrasperma*. *Ononis vaginalis*, on the other hand, was closer in its characteristics to the species *L.edulis* and *Tetragonolobus purpureus*. In conclusion, the present study might help researchers to better understand the classification of the a forementioned species.

Declarations

Author contribution statement

A. Huda Mohammed Abd-AlRazik Abusaief: Visualize and design experiences; Conducting experiments; data analysis and interpretation; Reagents, materials and analysis tools or contributing data; Books the paper.

B. Seham Hussein Boasoul: Conducting experiments

Funding statement

I extend my sincere thanks and appreciation to Professor Muhammad Asaad, Eshera (eshera@gmail.com) for his moral and financial support.

Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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