A taxonomic study of twelve wild forage species of Fabaceae

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ABSTRACT

Twelve species of wild leguminoseae 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, Tetrogonolobus 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, Tetrogonolobus 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.
of the legume species (Kahraman et al., 2014; Delgado et al., 2015; Ozkrahman 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-Akhadar, 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, 32° 47’ 012” N, 21° 59’ 052” E (Table 1). The species were identified according to the criteria set out by Jafri and El-Gadi (1986) and Boulos (1999). The specimens were preserved at the herbarium of the Department of Crop Science, Faculty of Agriculture, Omar Al-Mukhtar University, Tripoli, Libya. 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 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 magnification powers (Figure 1A), 2000 X (Figure 1B), showed the structure of the seed epidermal cells, power zoom 64 X (Figure 1C), showed the features of the epidermis, anticlinal walls, and outer periclinal walls. 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).

2.3. Methods of numerical taxonomy

Numerical taxonomy, known also as phenetic, 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 weighting 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, anticalinal walls, and outer pericalinal 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 (Figure 1B) & 4000 X (Figure 1C), showed the following: raised and depressed anticalinal wall, holed and toothed outer pericalinal wall, foveolate 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.

| Tribe      | Species                  | Site                        |
|------------|--------------------------|-----------------------------|
| Loteae     | Lotus edulis L.           | AllBaida, Gernada, Labraq   |
|            | Lotus ornithopodioides L. | AllBaida, Gernada, Shahat   |
|            | Tetragonolobus purpuratus| Moench, Gernada, Labraq, Shahat |
| Trifoliele | Medicago laciniata (L), MILL. Gard. Dist. | AllBaida, Gernada, Labraq |
|            | Medicago orbicularis (L), Bart. | AllBaida, Gernada, Labraq, Shahat |
|            | Medicago turbinata L.     | AllBaida, Gernada, Labraq, Shahat |
|            | Medicago polymorpha L.    | Labraq                      |
| Ononis      | Ononis vaginalis Vahl.    | AllBaida, Gernada, Labraq   |
| Vicieae     | Lathyrus aphaca L.        | Gernada, Shahat             |
| Vicia sativa L. | Gernada, Shahat       |
| Vicia peregrina L. | AllBaida, Gernada, Shahat |
| Vicia tetrasperma (L.) Schreb. | AllBaida                      |
Table 2. Description of 98 characters and character states for morphological and numerical analysis.

| Character State | 0 | 1 |
|-----------------|---|---|
| Plant duration  | annual | Perennial |
| Habit           | Foetid shrub | unarmed shrubs |
| Stems           | unwinged | winged |
| Branching       | at lower part only | at upper and lower |
| Thickness       | thick | thin |
| Color           | green dark | green light |
| Lea width       | wide (5 mm or more) | narrow (less than 5 mm) |
| Margination     | prominent | not prominent |
| Leaves          | simple | complex |
| Edge           | membranous | unmembranous |
| Arrangement     | regular | irregular |
| Rachis vesture  | hairy | glabrous |
| Flowers         | racemes, pod mostly curved | Pod straight flattened of 1-3 rounded segments |
| Pods            | septic | indehiscent |
| Stipules        | present | absent |
| Leaves          | with more than 3 leaves | simple |
| Sheath          | with more than 3 leaves | simple |
| Pedunculate     | present | absent |
| Leaf base       | Acute | Cuneate |
| Leaf blade      | cylindrical | compressed |
| Stipules edge   | membranous | unmembranous |
| Auricles        | present | absent |
| Corolla         | withered | yellowish cream |
| Flowers         | racemes, pod mostly curved | Pod flattened |
| Pods            | subglobose oblong-elliptic or circular | Pod flattened |
| Seed outline    | Ellipsoid shape | Discoid |
| Seed length     | From 1 cm or more | less than 1 cm |
| Mature stage     | Dehiscent | Indehiscent |
| Mature stage     | Dehiscent | Indehiscent |
| Seed outline    | Ellipsoid shape | Discoid |
| Seed length     | From 1 cm or more | less than 1 cm |
| Seed width      | less than 2 mm | more than 2 mm |
| Seeds surface   | shiny | dull |
| Seeds raised    | antennate | antennate |
| Seeds depression | antennate | antennate |
| Seeds grooved   | antennate | antennate |
| Seeds flatten   | antennate | antennate |
| Seeds grooved   | antennate | antennate |
| Seeds toothed   | antennate | antennate |
| Seeds foveolate | surface scan pattern | surface scan pattern |
| Seeds rugose    | surface scan pattern | surface scan pattern |
| Seeds scaleform | surface scan pattern | surface scan pattern |
| Seeds punctuate | surface scan pattern | surface scan pattern |
| Seeds reticulate | surface scan pattern | surface scan pattern |
| Seeds sulcate   | surface scan pattern | surface scan pattern |

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 2 (continued)

| Character State | 0 | 1 |
|-----------------|---|---|
| Corolla         | Petals free or wings adhering to the keel by a tooth | Keel very adherent |
| Flowers         | in terminal heads or axillary clusters, pod included in primrose calyx, 1-2 seeded | 0- present | 1- absent |

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

| Species | Characters | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---------|------------|---|---|---|---|---|---|---|---|---|----|----|----|
| L.e     |            | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  |
| L.o     |            | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  |
| T.p     |            | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  |
| M.l     |            | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  |
| M.o     |            | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  |
| M.t     |            | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  |
| O.v     |            | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  |
| L.a     |            | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  |
| V.s     |            | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  |
| V.p     |            | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  |
| V.t     |            | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1  | 1  | 1  |

(continued on next page)
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 mm² | 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 lacinata* (L.) Mill., Gard. Dict. | Reniform, oblong-ovoid | Yellowish-brown  | 2.2-3     | 1.4-1.4  | 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   | 1.6-1.7  | 2.73      | S      |
| 8   | *Ononis vaginalis* Vahl.             | Ellipsoid      | yellow-brown      | 1-2.2     | 1.1-1.5  | 2.08      | S      |
| 9   | *Lathyrus aphaca*.                   | Oblong         | Dark brown        | 2-3       | 2-4      | 7.5       | L      |
| 10  | *Vicia sativa* L.                    | Orbicular      | Yellow-brown      | 2.4-3.6   | 1.4-2.1  | 5.25      | L      |
| 11  | *V. peregrina*.                      | Spherical      | brown-black       | 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 pericllinal walls |
|-----|-------------------|-----------------------|------------------|-------------------------|
| 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 lacinata* (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*. | Fovulariate, Rugose | Raised, Depressed | Glabrous | Flattend, Holed |
| 10  | *Vicia sativa* L. | Fovulariate, Punctuate | Raised, Depressed | Glabrous | Grooved, Raised |
| 11  | *V. peregrina*. | Fovulariate, Punctuate | Raised, Depressed | Glabrous | Flattend, Grooved |
| 12  | *V. tetrasperma* (L.) Schreb. | Fovulariate, Scalariform | Depressed| 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. turbinata*.

3- Stipules coarsely toothed or lacinate; 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. lacinata*.

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. Foliate 3, corolla yellow, Features of epidermis fovulari ate 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 sub sessile; stipules sheathing *Ononis vaginalis*.

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

1- Leaflets 0.25-1.5 cm broad, oblong, oblivate, 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. lacinata* 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 antical wall cells varied among the studied *Lotus edulis* L., *Lotus ornithopodioides* L., *Tetragonolobus purpureus* Moench, *Medicago lacinata* (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. peregrina* L., and *V. tetrasperma* (L.) Schreb. The species *L. ornithopodioides* was morphologically close to all species studied of the genus *Medicago* at the 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...
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 attended anticlinal wall, raised and toothed outer periclinal wall, scalariform and reticulate coat scan pattern, and glabrous anticlinal wall texture.

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 110 X (Figure 2A) & 8000 X (Figure 2B), showed the following: raised and depressed anticlinal wall, holed outer periclinal wall, fovulariate and punctuate coat scan pattern, and glabrous anticlinal wall texture with bigger cells 2.196–2.914 μm wide.
Tribe 2. Trifolieae, Genus: Medicago, Medicago orbicularis. Seeds outline were as follows: length: 1.6–2.3 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 61 (Figure 5A) & 8000X (Figure 5B), showed the following: raised and grooved anticlinal walls, flattened and grooved outer periclinal walls, foveulate and rugose coat scan pattern, and glabrous anticlinal wall texture with bigger cells 4.831–5.095 μm wide.

Tribe 2. Trifolieae, Genus: Medicago, Medicago turbinata. Seed Morphology and Coat Scan outlines 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 70X (Figure 6A) & 8000X (Figure 6B), showed the following: raised and depressed anticlinal walls, flatter and holed outer periclinal walls, foveulate and punctuate coat scan pattern, and glabrous anticlinal wall coat.

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) & 8000X (Figure 7B), showed the following: raised and grooved anticlinal walls, grooved and raised outer periclinal walls, and foveulate and rugose coat scan pattern, and glabrous anticlinal wall texture.

Tribe 2. Trifolieae, Genus: Ononis, Ononis vaginalis. Seed Morphology and Coat Scan outlines were as follows: Seed length: 1–2.2 mm. 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 130X (Figure 8A), 2000X (Figure 8B), 4000X (Figure 8C) & 8000X (Figure 8D), showed the following: raised and depressed anticlinal walls, grooved and raised outer periclinal walls, foveulate and rugose coat scan pattern.
cultural 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

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**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 Scan outlines were as follows: Seed length: 2–4 mm. 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, foveolariate and rugose coat scan pattern.

**Figure 9.** Seed morphology and coat scan of *Lathyrus aphaca*. 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, foveolariate 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: 3–4 mm. 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, foveolariate 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.
L. edulis. whereas, Tetragonolobus purpureus the pod inflated, 5–8 mm diam., and the margins were bordered by 4 conspicuous undulate wings. Arambbari (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 distinguished the species is: orbicular; coat features: rugose and prolate, dimensions: 3mm. However, Patane and Gresta (2006) and Dudeja et al.
seeds, Tendrils well-developed; pod not constricted between the seeds.

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coat patterns compared to other seed-character groups. The differences with Fayed et al. (2019) which showed that the walls of 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 walls of Ononis vaginalis are raised, depressed. Our results are consistent with Fayad et al. (2019) which showed that Ononis vaginalis have raised and straight anticlinal walls, and convex outer periclinal 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- 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.peregrina 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.turbinata. Also, M.polymorpha was relatively similar to other species in its genus. The morphological description results indicated that the two most similar species were Ledulis and Tetragonolobus purpureus. The species Lornithopodioides 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 Ledulis 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

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Additional information

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