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RESEARCH PAPER

Comparative Anatomical Study of Fruit and Seeds of Six Species within Selected Six Genera of the Family Fabaceae Lindl. In Iraq

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A B S T R A C T

The fruit and seeds of six species within six genera belonging to the Fabaceae family were studied. The paraffin method was applied to microscopic studies. The study showed different fruit outline shapes and the fruiting layers varying among them, however, all fruits consisted of three layers the exocarp, mesocarp, and endocarp. The thickness of pericarp layers was different in all taxa. Although the shape of seeds was different among all studied taxa except *O. galegifolia* and *T. echinatum* they were similar, the seed contents were examined as well, the testa tissue was described and the embryo shape and position were determined, where *O. galegifolia* was the single species devoid of macrosclereids.

KEY WORDS: Anatomy, Fabaceae, Macrosclereids, Pericarp, Testa.
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1. INTRODUCTION

The third largest family of flowering plants is Fabaceae possess more than 1820 species belong to 643 genera, most of these species are important such as food. The Fabaceae consist of herbs, shrubs, trees, or vines, with spines sometimes present. The roots of many members have a symbiotic association with nitrogen-fixing bacteria (*Rhizobium* spp). The Fabaceae family is traditionally classified into three subfamilies (sometimes treated as separate families); *Caesalpinioideae*, *Mimosoideae*, and *Faboideae* (Townsend and Guest,1974; Simpson, 2006; Weakley, 2007). The family is specified by its distinctive characteristics of flowers and fruits (Metcalfe and Chalk 1957), for this reason, compared the fruits of the family with many of the fruits of other families. The importance of the family fruits refers to the developmental signs of the family, and it is also fateful to the mother of the Bean and Pea family (Judd et al., 1990; APG III, 2009).

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A large number of species economically important, it is used as food and fuel, as a source of mineral ores, and as an interested source of atmospheric nitrogen when the ratio is fixed in the earths cover (Brouwer, 1962; Poorter & Nagel, 2000; Mirzaei *et al*., 2015; Al-dabbagh and Fathulla, 2022). The third largest family of flowering plants, most species of the Fabaceae family are important such as food, fodder, wood, ornamentals, and raw materials for industry. Before germination the woody legume seeds which require treatment because of poses impermeable seed coats, as it prevents water uptake, gaseous exchange and radicle emergence (Zohary, 1946; Von Denffer *et al.*, 1971; Stace, 1980; Simpson, 2006; Mirzaei *et al.*, 2015; Toksoy *et al*., 2015). As for the Fabaceae family, it is represented by about 50 genera and more than 300 marine species, as well as a number of farmed species (Al-Dubaisi, 2008). The fruits are of taxonomic importance in this family and in other plant families because of the changes that can be used to distinguish between different plant species and because they are also among the least vulnerable to environmental changes. Through the

foregoing, the delivery number is in an exhibition of great categorical importance. However, the Fabaceae family did not get the deserved studies in Iraq (Al-Dubaisi, 2016). In the Fabaceae family the shapes and types of fruit cells, make them of taxonomic importance even among closely related species (Al-Blesh and Al-Dulaimi, 2020). At last decade some previous researchers were collected the plants and preserved them in some Iraqi herbaria, such as Zohary (1946) mentioned the distribution of the species in Duhok, the plants are sub-Mediterranean (Irano-Turanian Region) elements. Al-Rawi (1964); Townsend and Guest (1974) cited, the most members of Fabacea are common as wild and range plants in Amadia, Rawanduz and Persian Foothill districts of Kurdistan-Iraq, they naturally grow up to altitude 2300m. Ridda and Daood (1982) pointed out that the species of the family are distributed in Amadia, Rawanduz, Sulaimaniya, Nineveh, Arbil, and Kirkuk districts in Iraq.

According to (Townsend and Guest, 1974) the number of distributed species of the selected genera in Iraq were *Astragalus* L., 115spp. (128 taxa); *Cicer* L., 5spp.; *Lens* Mill., 3spp.; *Medicago* L., 17spp. (24 taxa); *Onobrychis* Mill., 14spp.; *Trifolium* L., 33spp. (38 taxa).

Anatomical characters are widely used in many aspects of taxonomy (Davis and Heywood, 1973), and provide valuable information which can be employed for classification, in addition to the having a large role in the plant identification (Metcalfe and Chalk, 1957).

The current study is focusing on the comparative anatomical study for fruit and seeds of selected six species of the family Fabaceae, and applying an anatomical study of cross sections of fruit and seeds, in order to determine the main anatomical variations among these naturally distributed six genera species in different geographical areas in Iraq.

2. MATERIALS AND METHODS

The following steps were applied for the paraffin method to prepare the permanent slides. The plant materials were taken from the mature fruits of the following species, where each species represents the genus it belongs to: (table 1)

1. *Astragalus hamosus* L. for the genus *Astragalus* L.
2. *Cicer pinnatifidum* Faub. *et* Sp. for the genus *Cicer* L.
3. *Lens orientalis* (Boiss.) Hand. -Mazz. for the genus *Lens* Mill.
4. *Medicago laciniata* (L) Mill. for the genus *Medicago* L.
5. *Onobrychis galegifolia* Boiss. for the genus *Onobrychis* Mill.
6. *Trifolium echinatum* M. Bieb. for the genus *Trifolium* L.

Dry fruit samples were put in (NaOH) solution (1-3% molarities) for 24 hours, washed with distilled water (D.W), then fruit samples were cut in a size of 1cm, and kept in a small vial containing FAA solution (5ml Formalin; 5ml Glacial acetic acid; and 90ml Ethanol alcohol %70) at room temperature for 24 hours (Al-dabbagh and Saeed, 2020). The samples were dehydrated by placing in a series of ascending concentrations of ethanol 50%, 70%, 80%, 90% and 100% for 2 hours in each concentration. The samples were immersed in a mixture solution of absolute alcohol and xylene in three different ratios of alcohol: xylene (3:1, 1:1, and 1:3) then in absolute alcohol-xylene, and lastly in xylene for 0.5 – 1 hour. Finally, the samples were placed in a mixture of melted paraffin and xylene 1:1 overnight at 60Co. The paraffin block sections were done by rotary Microtome (Silver). The sections were stained by using Safranin (100ml D.W with 1gm safranin) and fast green (100ml %70 ethanol with 1ml fast green), eventually, examined by Olympus light microscope, the photographs were taken by the mounted digital camera on the microscope.

3. RESULTS

3.1 Fruit outlines: (fig. 1) and (table 2)

The fruit outlines displayed that there were great differences among the cross sections of the fruits in the studied taxa. The fruit in *A. hamosus* was ovoid oblong contains an oblong seed almost with two furrows making a waist at the end of one side. While, the fruit in *C. pinnatifidum* was semi-circular contain one irregular seed. The fruit in *L. orientalis* was ellipsoid contains semi-circular with a hump in one side seed. Whereas the fruit in *M. laciniata* rectangular contains oblong seed. The fruit outline in *O. galegifolia* was comprises of two parallel ellipsoids, each part contains a semi-circular seed. *T. echinatum* fruit has irregular oblong shape contains semi-circular seed.

3.2. General description of fruit cross sections:

In general, the cross sections in the studied species showed three different layers were epidermis (exocarp), and two perpendicular

sclerenchyma layers (mesocarp and endocarp) under it.

Table 1: Specimens* of the studied Fabaceae taxa with their information.

Data / Taxon	Collection data			Specimen No.	Habitat
	Location and District	Date	Alt. (m)		
A. hamosus	Sulaimaniya (MSU)	25.5.2006	737	4768	Clay and sandy soils
C. pinnatifidum	East of Amadiya (MAM)	28.5.2016	1187	4801	In Limestone, in the shade of *Pistacia* and *Quercus* forests,
L. orientalis	Hawraman M. East of Penjwin (MSU)	1.6.2016	1560	4823	Limestone and rocky clay
M. laciniata	N.W. of Fallujah (LCA)	6.4.1961	43	00186	Loamy soil in plain
O. galegifolia	N.E. of Shekhan (MAM)	28.5.2016	530	4826	Loam-clay soils in plains
T. echinatum	On top of Maqlub M. (FNI)	9.5.1984	1055	2823	Sandy stone and rocky clay

* Specimens from Herbarium of College of Science, Salahaddin University-Erbil, Iraq (ARB).
(MAM): Amadya District, (MSU): Sulaimaniya District, (FNI): Nineveh District, (LCA): Central Alluvial Plain District.
(m): in meter.

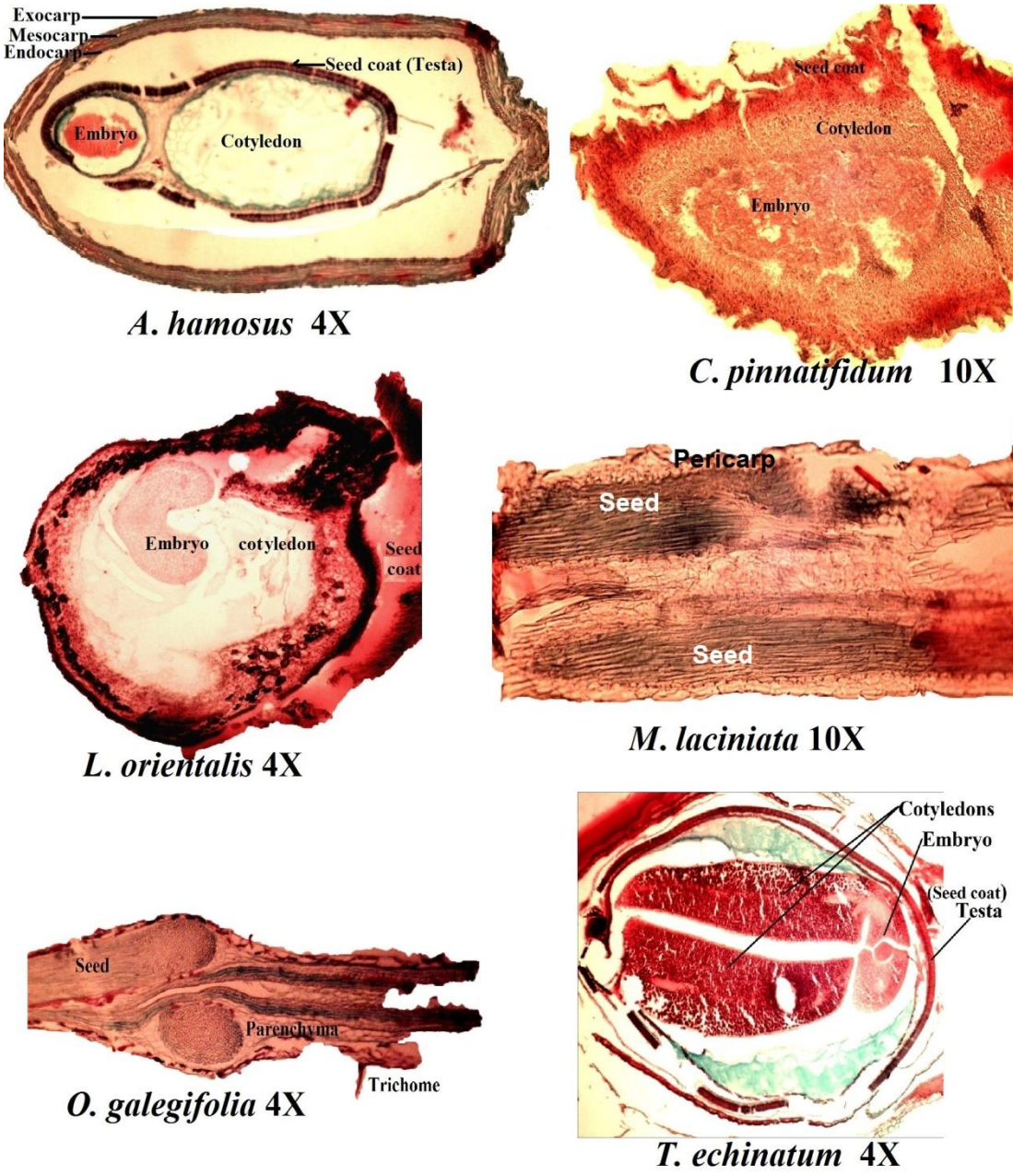

Figure 1: Variations of fruit cross-section outlines of studied Fabaceae species

3.2.1. *A. hamosus*: (fig. 2) and (table 2)

The epidermis consists of a single layer, one oblong cell thick, and covered with a thick layer of cuticle wax. The epidermis contains stomata as well. Followed by 3-4 layers of lengthy sclerenchyma beneath it. Then sclereids of two cells thick, show as the subspherical lumen.

The testa (seed coat) consists of a layer of macrosclereids, and then comes 2-3 layers of sclerenchyma fibers, there was parenchyma tissue around the small embryo and large cotyledons.

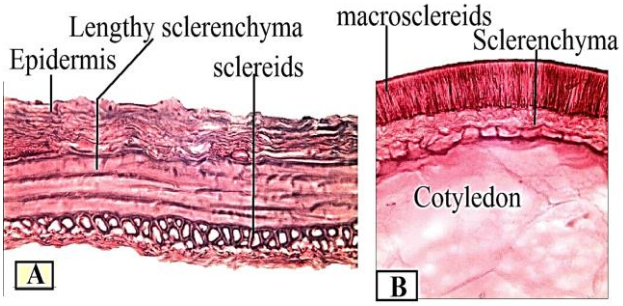

Figure 2: Magnified portions of fruit and seed of *A. hamosus* species. A- Pericarp (40X) B- Seed (40X)

3.2.2. *C. pinnatifidum*: (fig. 3) and (table 2)

The epidermis consists of a single layer contain stomata, and covered with a thick layer of cuticle wax. Underneath, 9-10 layers of lengthy sclerenchyma were detected. Then, 3-4 cells thick of sclereids in a subspherical shape were identified, containing abundant solitary prismatic crystals.

The testa (seed coat) consists of 5-6 layers of sclerenchyma fibres, and a layer of macrosclereids. There was parenchyma tissue around the embryo and cotyledons.

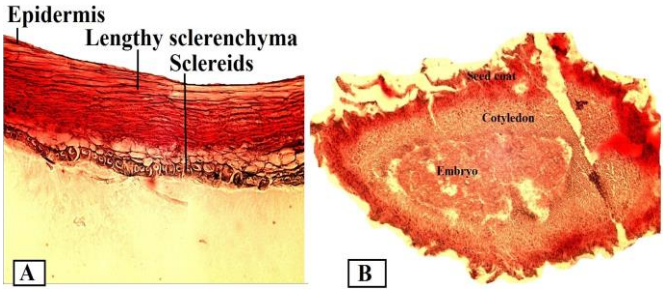

Figure 3: Magnified portions of fruit and seed of *C. pinnatifidum* species. A- Pericarp (40X) B- Seed (10X)

3.2.3. *L. orientalis*: (fig. 4) and (table 2)

The epidermis consists of a single layer contain stomata, and covered with a thick layer of cuticle wax except for stomata areas. Beneath the epidermis 5-9 layers of lengthy sclerenchyma were observed. Then, sclereids of 5-6 thick cells in a sub spherical shape and very thick walls were noticed.

The testa (seed coat) consists of a layer of macrosclereids, under the testa there were 6-7 layers of sclerenchyma fibers. The testa surrounds the curved embryo and proteinous cotyledons.

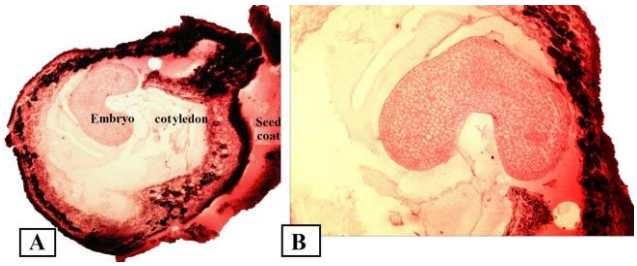

Figure 4: Magnified portions of fruit and seed of *L. orientalis* species. A- Pericarp (4X) B- Seed (10X)

3.2.4. *M. laciniata*: (fig. 5) and (table 2)

The epidermis consists of a single layer contain stomata, and covered with a thick layer of cuticle wax. Beneath the epidermis, 9-10 layers of lengthy sclerenchyma, containing abundant solitary prismatic crystals were seen. Then sclereids of 5-6 cells thick, show as the subspherical in shape with very thick cell walls.

The seed features were not clear.

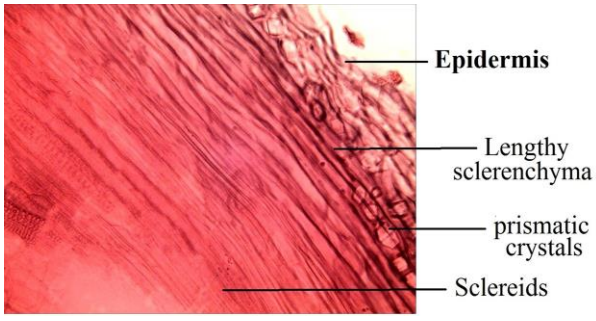

Figure 5: Magnified portions of fruit of *M.laciniata* species. Pericarp (40X)

3.2.5. *O. galegifolia*: (fig. 6) and (table 2)

The epidermis involves a single layer containing stomata, and covered with a thick layer of cuticle wax, the epidermis contains non-

glandular unicellular trichomes. Beneath the epidermis sclereids of 3-5 cells thick, show as the subspherical in shape with very thick cell walls. Then 3-4 layers of lengthy sclerenchyma, contain solitary prismatic crystals, the mass of parenchyma tissue was surrounding the embryo and cotyledons.

The testa (seed coat) consists of 5-7 layers of spherical sclereids, under the testa there were the embryo and proteinous cotyledons. In seeds of *O. galegifolia* the layer of macrosclereids was absent.

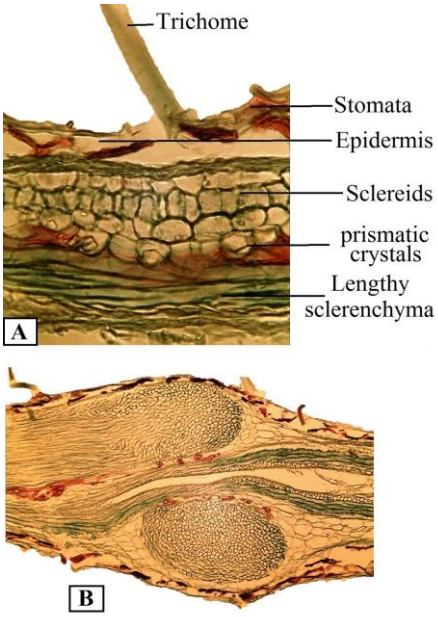

Figure 6: Magnified portions of fruit and seed of *O.*

3.2.6. *T. echinatum*: (fig. 7)

The epidermis consists of a single layer covered with a thick layer of cuticle wax. The epidermis involved abandoned sessile papillae and unicellular trichomes with stomata. Beneath the epidermis, there were 6-7 layers of lengthy sclerenchyma, contain abundant solitary prismatic crystals. While the sclerenchyma subspherical in shape with very thick cell walls, gathers in the form of semi-circular clusters, forming clear protrusions on the edges of the fruits.

The testa (seed coat) consists of single layer of macrosclereids, followed by 2-3 layers of spherical sclereids, under the testa there were the straight embryo and large proteinous cotyledons.

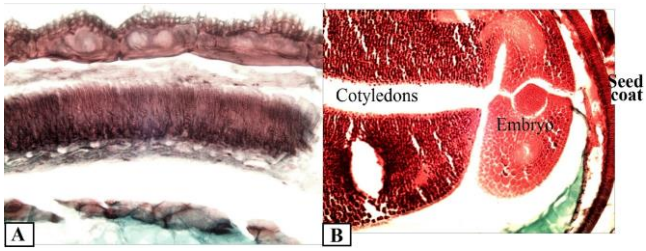

Figure 7: Magnified portions of fruit and seed of *T. echinatum* species. A -Pericarp (40X) B- Seed (10X)

Table 2: Anatomical comparison of fruit traits in the studied taxa.

traits / Taxon	Fruit outline shape	Seed shape	lengthy sclerenchyma layers	Sclereids layers	Sclerenchyma layers of testa	Macrosclereids
A. hamosus	ovoid oblong	oblong almost with two furrows making a waist at the end of one side	3-4 layers	2 layers	2-3 layers	present
C. pinnatifidum	semi-circular	irregular	9-10 layers	3-4 layers	5-6 layers	present
L. orientalis	ellipsoid	semi-circular with a hump in one side	5-9 layers	5-6 layers	6-7 layers	present
M. laciniata	rectangular	oblong	9-10 layers	5-6 layers	----	----
O. galegifolia	two parallel ellipsoids	semi-circular	3-4 layers	3-5 layers	5-7 layers	absent
T. echinatum	irregular oblong	semi-circular	6-7 layers	Variant with location	2-3 layers	present

4. DISCUSSION

The results of the cross-section outlines gave a high taxonomic value to separate the understudied species. These differences are probably due to the morphological structure of the of the fruits of the studied species. In the species *A. hamosus*, *C. pinnatifidum* and *T. echinatum*, which were characterized by semi-circular and elongated circular shapes, due to the inflated of the fruits of these species. The other species, such as *L. orientalis*, *M. laciniata* and *O. galegifolia*, their rectangular shapes return to their flat fruits.

There is no microscopic examination of the fruits done by (Metcalf and Chalk, 1957), seeing that they were only concerned with the anatomy of vegetative organs. Although, Fahn

(1982) and Esau, (1965) explained the fruits of monocots and dicots anatomically in a comprehensive manner without specifying families and genera, some terms have been taken and proven through it. Accordingly, the internal characteristics of fruits and seeds were described based on some recent researchs, such as (Jacobs, *et al*., 2010; Steeves and Sawhne, 2017; Al-dabbagh and Saeed, 2020).

The epidermis was single layer and contains stomata in all studied species. Whereas the epidermis of *O. galegifolia* comprises the unicellular non-glandular hairs, and the species *T. echinatum* was distinguished by the presence of unicellular papillae hairs on the epidermis, as proven by (Al-Otraqche, 2022).

The sclerenchyma layers differed between species, where the two types *C. pinnatifidum* and *M. laciniata* recorded a high number of lengthy layers were between 9-10 layers, and the lowest layers were about two layers recorded in the species *A. hamosus*. *O. galegifolia* was unique in the presence of the circular layer above the lengthy layers unlike other species, as explained by (Al-Blesh and Al-Dulaimi, 2020). Prismatic crystals appeared in most of the studied species except the two species *A. hamosus* and *L. orientalis*, where their presence was not observed.

The seeds of the studied species almost were similar in shape, except for the species *A. hamosus*, which was distinguished by the presence of two grooves between the embryo and the cotyledons. All species are characterized by having the seed coat of a single layer of macrosclereids except *O. galegifolia* the seed coat is made up of 5-7 layers of sclerenchyma. Nonetheless, the number of sclerenchyma layers under testa varied, with a thickness of two layers in *A. hamosus* as a minimum and a thickness of 7 layers in *L. orientalis* as a maximum. The microscopic study observed two types of the embryo, which were curved as in *L. orientalis* and straight as in *T. echinatum*.

5. CONCLUSION

The current study has reached the following conclusions:

The genera were varied in fruit shapes. The fruits in the studied species of the six genera are comprised of three different layers. There are some anatomical variations that have a significant role in species delimitation.

This work focused on some species within some genera of the Fabaceae family, along with the previous work and other tasks that would be carried out on the genera of this family, they could contribute significantly to enriching the Iraqi flora by constructing an anatomical taxonomic key for separating the genera of the family in the future.

Conflict of Interest (1)

There is no conflict of Interest.

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