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ORIGINAL ARTICLE

A COMPARATIVE ANATOMY AND MORPHOLOGY STUDY OF SEEDS BY SEM FOR SOME SPECIES OF *LATHYRUS* L., 1753 (FABALES, FABACEAE) IN IRAQ

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ABSTRACT

Anatomy and morphology study of seeds for seven *Lathyrus* L., 1753 species in Iraq; the species were: *L. annuus* L.,1753, *L. aphaca* L., 1753, *L. chloranthus* Boiss., 1859, *L. cicera* L., 1753, *L. sativus* L., 1753, *L. inconspicuus* L., 1753 and *L. sphaericus* Retz.,1753. There are 17 species of the Fabaceae family that are distributed throughout the middle and northern regions of Iraq. Using a scanning electron microscope, the study's characteristics were examined to ascertain the taxonomic relationships, interactions, and issues that impeded the species' ability to be separated according to taxa.

The seeds were glossy in all species except *L. sativus* L. and *L. sphaericus* L. which were matt, the surface cells wall undulation, the wax covered the peak of the conical papillae, the anatomical characteristics determined by cross section in seeds and pointed some trails such us ,Epidermis parenchyma thickness and Palisade, the species *L. annus* L. and *L.aphaca* L. were given the highest value of exocarps thickness (5.5) µm while the species *L.sativus* recorded the Lowest mean (3.8)µm, the seed coat in *L. sativus* L. is detached from some epidermis in some area with pit aperture, in according the epidermis thickness, the species classification to three groups the *L. aphaca* L. and *L. sphericaus* Retz. recorded the highest value (46-49) µm respectively, *L. sphaericus* Retz. recorded the highest value of palisade thickness which was 61 µm, some unique trails such us present the pit in exocarp, and the pit cavity was along parenchyma layer in *L. sativus* L., the similar morphology features are considered the evidence for the proximity of the taxa, so the internal structure (anatomy) of the seeds can be used to separate the taxa.

Keywords: Anatomy, Fabaceae, *Lathyrus*, Mesocarp, Seed, SEM

INTRODUCTION

Fabaceae is a large and economic family, it is known as the pea, legume, bean family, and it is regarded as the third greatest group of flowering plants, following the Asteraceae and Orchidaceae families, which include 730 genera and over 19,400 species in the world. It is

divided into three subfamilies, Caesalpinioideae, Mimosoideae and Papilionoideae, with a total of 800 different genera and 20,000 distinct species (Lewis *et al*., 2005).

The taxonomic group known as Papilionoideae encompasses the majority of cultivated plant species, and it is important because it has a worldwide distribution and consists of annual or perennial herbs and trees (Naik and Deshpande, 2021). The variation in the seed shapes and sizes reflects the differentiation in develops (Zuk *et al.,* 2014). In angiosperm, seed develops from the ovule after it is fertilized, the seed consists of the embryo (Bradford and Nonogaki, 2009), and the sporophyte layer of the ovule primordium is where the seed coat originally formed (Schneitz *et al*., 1997).

The morphology characteristics are considered important in determining the species because the seeds have many variations in characteristics used in identification like, size, color pattern, general shape, hilum width, and length (Zohary *et al*., 2012; Güneş, 2013). In legumes, the first development happened in the seed coat and endosperm and then in the embryo (Weber *et al*., 2005); the seed features in legumes support the concept of one family Fabaceae as advocated already by De Candolle (1825). The form and substance of the seed coats vary widely throughout species; however, the Fabaceae share a fairly common seed structure blueprint (Lush and Evans, 1980). Parenchyma cells constitute the inner most region of the seed coat, because of its role during embryonic development, some researchers refer to the parenchymatous region as the "nutrient layer, parenchyma cells in the seed coat during maturation lose the proplast and the inner layer is crushed (Van- Dongen *et al*., 2003).

The subfamily Papilinoideae consists of many genera, such as *Astragalus* L. , *Vicia* L., *Cicer* L. , *Pisum* L., *Lathyrus* L. and other , *Lathyrus* a genus the consists of many species 17 in Iraq (Townsend and Guest,1974). *Lathyrus* belongs to the subfamily Papilionoideae known as sweet pea. The species is herbaceous, perennials in the temperate region, the common species is *Lathyrus sativus* L. which refers to the genus, the members are distributed in the northern hemisphere with a disjunction in south America, and distributed and in Western Eurasia, specially around the eastern Mediterranean, with over a third of the species native in Turkey (Davis, 1970). Many researchers studied the seeds of Fabaceae, Quilichini *et al*., (2022) pointed out that the seed coat is a good source of dietary fiber, as well, the previous studies such as Kislev and Hop (1985) and Güneş (2013) have indicated that relying just on seed traits is insufficient for distinguishing across taxa. However, when combined with broader morphological criteria, the use of seed characters may prove beneficial in distinguishing some species; Abou-El-Enain *et al*., (2007) have examined the morphology of 34 *Lathyrus* taxa that were collected from seed banks in 18 different nations. However, they only provided information on the papillae qualities; they did not specify whether the seed surface was smooth or reticulate. Güneş (2013) studied the seed textures for some taxa of this genus in Turkey and pointed out that species have a wide variation in characters, which may help to determine the species. Also Aloush (2014) studied the taxa of *Lathyrus*, which spread in Iraq, including the morphological characteristics of pods and seeds such as dimensions, hilum shape and length, and color. The researcher pointed out that the species has a wide variety of seed characteristics may be helpful in determining the species, the advanced

techniques such as SEM has brought benefits for the investigation of seeds, to the best of our knowledge, no prior research has been done on *Lathyrus* species seeds in wild plants in Iraq.

As a result, the primary objective of this paper is to identify the morphological, anatomical, and testa structures of seeds from seven different species and use these characteristics in the identification process.

MATERIALS AND METHODS

The fresh specimens and their seeds were collected from their natural habitats at flowering, at the beginning of March. The survey site included many regions in the middle and north of Iraq, which comprised the mountainous regions (Amadiya district MAM, Rowanduz district MRO, Sulaimaniya district MSU), and upper Jazira district (Nineveh district FNI, Arbil district FAR, Persian foothills district FPF). The dry specimens were stored in the Baghdad, Iraq, College of Science, Baghdad University, (BUG) herbarium.

Morphological investigatione dependened on many characters, including stem, leaflet, flower, pod and seed, the identification of the taxa was based on the (Davis, 1970; Townsend and Guest, 1974; Lewis *et al*., 2005). The morphological characteristics of the seeds (general shape, ornamentation on the seed surface) were examined by a scanning electron microscope. Ripe seeds (2-3) were mounted into stubs with double-side adhesive tape and then coated with the seed on the gold coat; pattern was examined lateral surface of the seed. Anatomical investigations were performed using fresh samples kept in five ml of formaldehyde 40%, 90ml of alcohol ethanol 70% and five ml of acetic acid. The paraffin wax method was applied for preparing cross sections of seeds, the sections were stained with safranin and fast green (Johansen ,1944). Characteristics pointed after cross section in seeds by microtome then measured, exocarp, epidermis, palisade layer and endosperm layer thickness. The terminology in this study is based on (Güneş, 2013).

RESULTS

SEM Results

Morphology testa appearance variation between species, asymmetric, the shape was oval - globose in species *L. annuus*, *L. aphaca*, *L. chloranthus* while it semi- triangular in *L. sativus* semi triangular - triangular in *L.cicera*, oblong in *L. inconspicuus* and semiovate in *L. spharicus*. According to SEM the result showed a wide variation in the morphological characters (Pl.1).

Lathyrus annuus L., 1753: Seed glossy because it was covered with wax, sculpture seemed (reticulate), and tuberculate ; papillae were reflat or dished, and it seemed homogeneity in SEM or irregular; the anticlinal walls of epidermis cells were clear, thick compound, cell borders , and cell wall wavt.

Lathyrus aphaca L., 1753: Seeds are smooth, semi-matt, irregular, multi-ribbed, anticlinal walls sunken, and thick, surface covered by wax, surface without papillae-like favulariate.

Lathyrus chloranthus Boiss., 1859: Glossy, papillae arranged regularly seem like conical peaks, top of peak flat covered by wax, anticlinal walls sunken, thick, and undulation.

Lathyrus cicera L., 1753: Ribbed, anticlinal walls of epidermal cells flattened to sunken, surface cell uniformity, glossy and smooth, yet at the same, time the seed surface sculpture has a conical peak, top of which is coated with a wax that collects at top and a star – like shape

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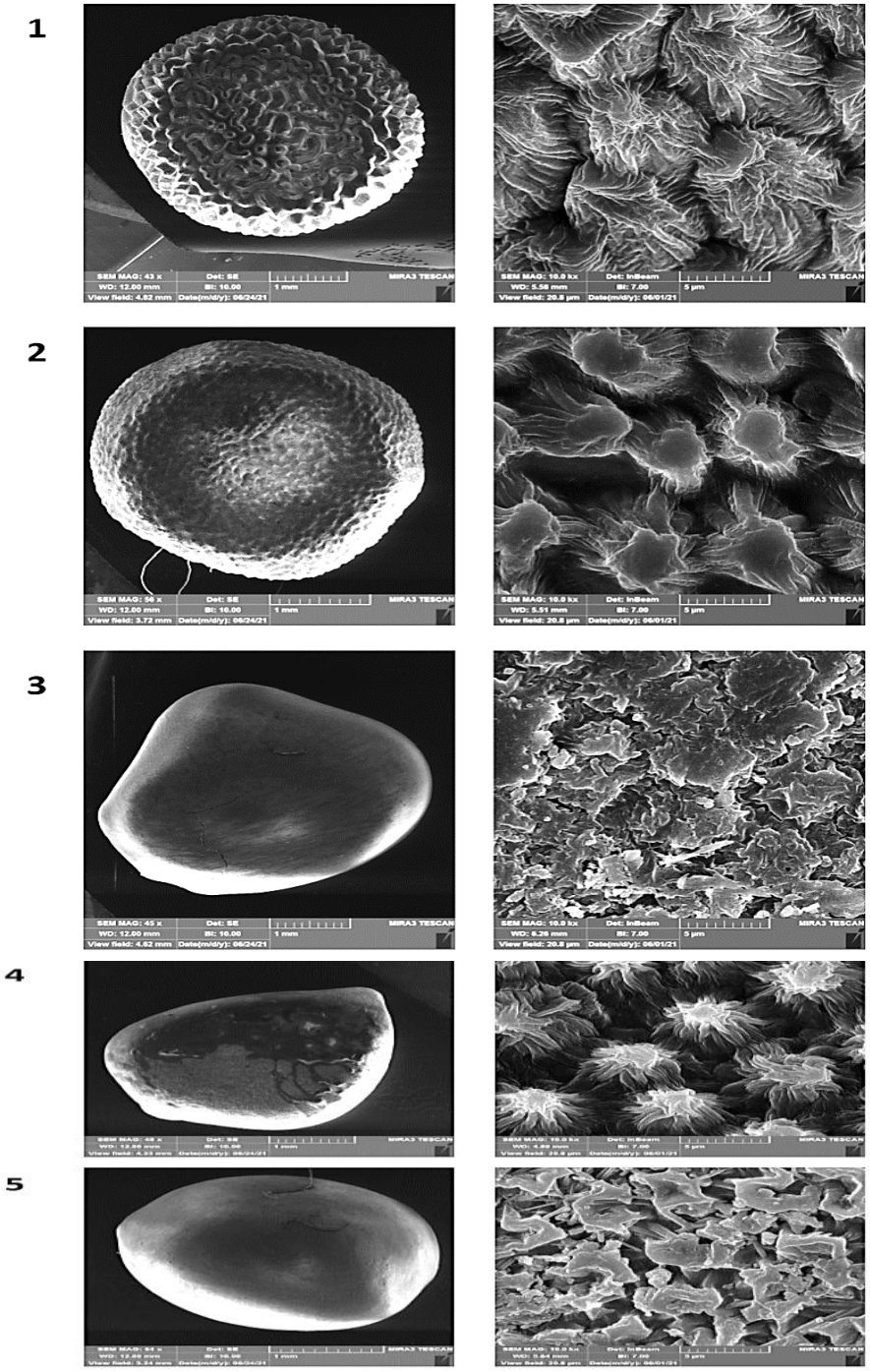
Lathyrus inconspicuus L., 1753: Matt, many ribbed, anticlinal walls raised thin, the wax slightly cell wall surface homogeneity.

Lathyrus sativus L., 1753: Semi-matt, smooth, the anticlinal walls, irregular, some of them large, and the other small, thin wall, raised spreads lightly on ridges.

Lathyrus sphaericus Retz., 1753: Matt, smooth, anticlinal walls thick, ribbed, irregular, the surface cell highly represented and seemed like rods and needles on the surface of the seed.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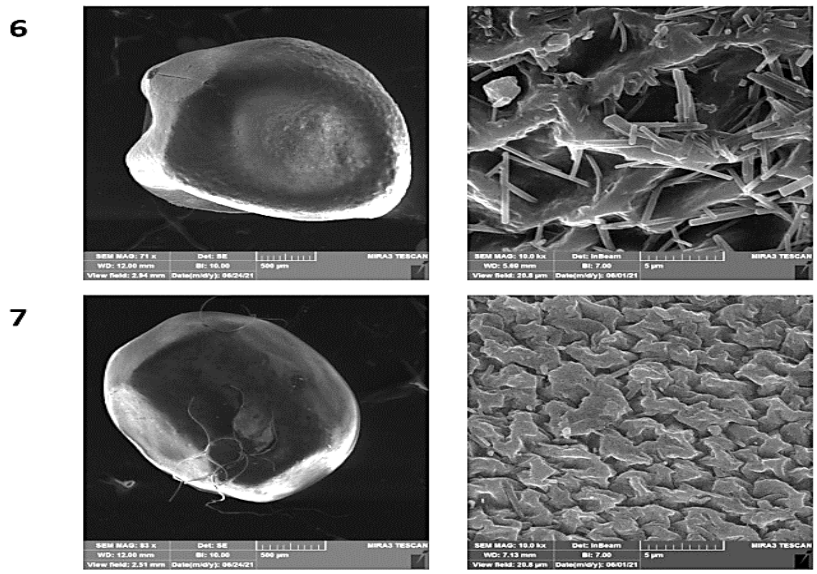

Plate (1): Seed sculpture of examined taxa (SEM); (1) *L. annuus*, (2) *L. chloranthus*, (3) *L. sativus*, (4) *L. cicero*, (5) *L.aphaca*, (6) *L. sphaericus*, (7) *L. inconspicuous*.

Cross section results

Seven species were studied, in all species investigated layers of cuticle (2-3)µm. The result showed variation between species, mainly in the patterns of differentiation modification and dimensions of the cross section layers (Tab.1; Pl.2-7). The shape of the exocarp in the cross section was undulate in *L. annuus*, *L. chloranthus*, *L. cicero*, *L. sphaericus* and *L. sativus*.

The exocarp (seed coat or testa): According to the thickness of the testa, the species *L. annuus* and *L. aphaca* gave the highest value of 6.2 µm, so as *L. chloranthus* and *L. sphaericus* which give a similar mean (5.5 µm), and the thickness of the exocarp in *L. cicera* was 5.2µm, while the species of *L. sativus* and *L. inconspicuous* were recorded the lowest mean (3.8-4.7 µm) respectively. The result showed that the seed coat in the species *L. sativus* is detached from the sub epidermal in some areas with a pit aperture, present at the end of the pit cavity when its junction with the cavity of the cell. Also the pit cavity is along the parenchyma layer, and there is a tracheid bar. In addition, that there is a counter palisade touch with the seed coat species. *L.cicera* is distinguished in trichomes spread along the surface of the seed coat (testa), legume seed characters support the taxonomy of the different taxa in the family (Fabaceae) and genus *Lathyrus*, as advocated already by De Candolle (1825). In lower part of the seed coat, the closely associated outer layer of endosperm is known as the aleurone.

The epidermis: There are differences among the species that showed varying values, and the species could be categorized into groups based on the thickness of their epidermis.

In the first group, this group's mean epidermal thickness ranged from (16–24) µm. The species that recorded the lowest value were *L. annuus*, *L. chlorantus*, and *L. sativus*, all of which belong to the same section (sativus) in the *Lathyrus* genus. The second group consisted of *L. cicera* and *L. inconspicuous*, whose epidermis thickness was measured between 28 and 36 µm. The third group consisted of *L. aphaca* and *L. sphaericus*, which recorded the highest mean of 46-49 µm. As a result, the species *L. annuus* recorded the thinnest epidermis (16.3 µm), while the species *L. aphaca* recorded the thickest epidermis at 48.7µm in comparison to other species.

Mesocarp (palisade): Below the epidermis, there are palisade layers, which include elongated sclereids called Macrosclereides, Malpighin cells, and palisade cells. There are many regional palisade cell walls separating terminal caps from their basal parts (Smýkal *et al.*, 2014). In cross section, the palisade layer is called the Light Line or Linea Lucida (Hamly, 1935). The highest value of palisade thickness recorded by *L. cicera* was (60.8) µm, while the lowest value was recorded in *L. sativus* (35.6) µm. Species are classified into groups based on their thickness.

The first group: in this group, the palisade thicknesses ranging from 36 to 41µm, and it includeds *L. sativus*, and *L. inconspicuous*. The second group: included the species *L. annuus*, *L. aphaca*, and *L. chloranthus* which recorded thickness from 45 to 51µm. The third group: contained *L. cicere* and *L. sphaericus*. In this group, the palisade thickness was between 53 and 61µm, and it's considered the highest thickness, current study pointed out important characteristics such as the osteosclereides distinguished in *L. sativus* below the palisade layer, and its differentiation almost from the sub-epidermal layer and also considered a major cell. During this test, a differentiation where cell death was detected and followed by parenchyma and macrosclerides (Ranathunge *et al*., 2010).

Endosperm: The inner of parenchyma contact with endosperm, there is wild variation in endosperm layer thickness between species, the species can be divided into groups according to the endosperm thickness.

First group: The thickness value ranged from 34 to37 µm. This group contained the species *L. aphaca* , *L. chloranthus* , and *L. inconspicuus* , and it is considered the lowest value. Second group: endosperm thickness ranging between 46 and 491µm, included the species *L. annuus*, *L. cicera*, and *L. sphaericus*. Third group: represented by species *L. sativus* with the highest value (77.5)µm, the result showed that the storage parenchyma tissue, and the parenchyma were connected with the endosperm, and it was called the nutrient layer owing to pit function during embryo.

Table (1): Anatomical characters of the seeds in examined taxa (in µu).

Taxa	Exocarp thickness	Epidermis thickness	Palisade layer thickness	Endosperm layer thickness
L. annuus	5.5-6.6 (6.2)	12.5-17.4 (16.3)	40.1-50.2 (45.3)	40.1-49.5(47.6)
L. aphaca	6.1-6.8 (6.2)	40.9-51.2 (48.7)	43.2-50.7 (49.6)	33.6-39.7(38.1)
L. chloranthus	4.5-6.2 (5.5)	15.5-19.4 (17.3)	46.1-57.3 (50.7)	33.7-38.1(36.2)
L.cicera	4.5-5.6 (5.2)	30.6-37.4 (35.4)	55.5-65.4 (60.8)	44.5-51.7(48.3)
L.inconspicuus	3.5-4.1 (3.8)	33.2-39.1 (35.1)	39.2-44-6 (40.1)	30.1-39.7(33.6)
L. sativus	3.5-6.1 (4.7)	20.1-26.4 (23.9)	30.1-39.4 (35.6)	70.1-79.6(77.5)
L. shaericus	5.2-6.1 (5.5)	40.2-49.7 (46.3)	48.6-55.7 (53.9)	40.1-49.6(47.5)

*The numbers in the left point to the lower limit while the number in right point to upper limit, the numbers between bracktes point to the average.

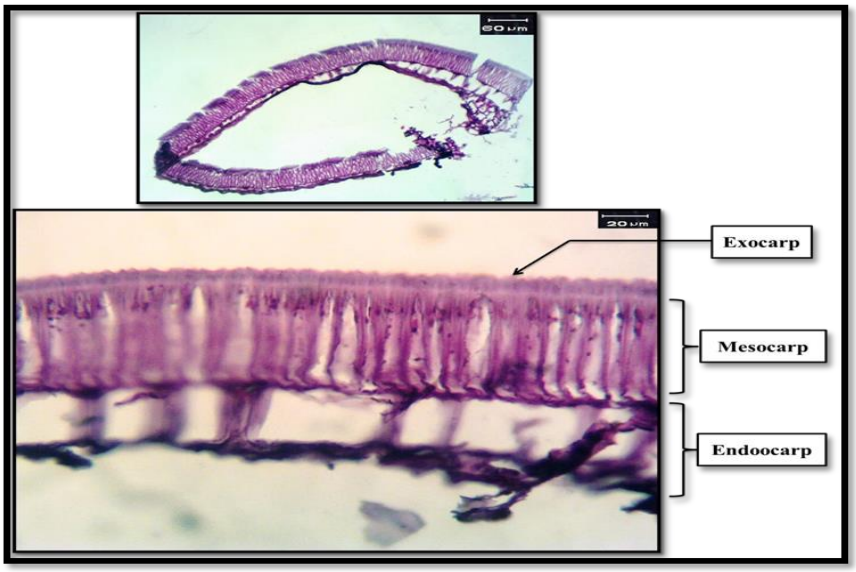

Plate (2): Cross section in the seed of *L. annuus* .

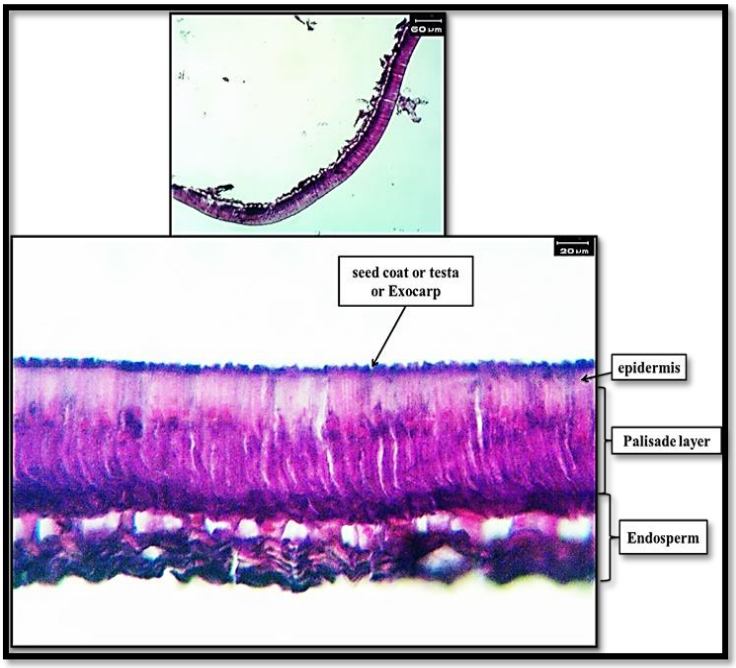

Plate (3): Cross section in the seed of *L. chloranthus.*

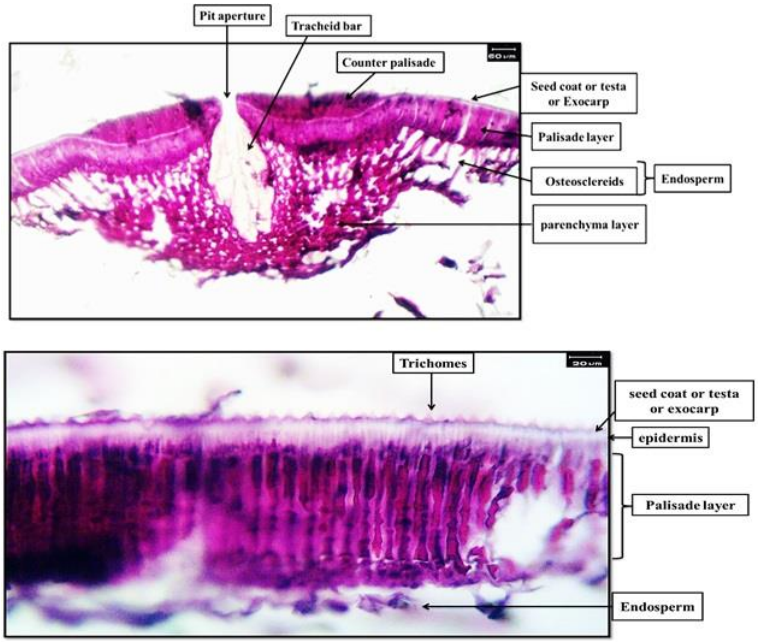

Plate (4): Cross section in the seed of *L. sativus*.

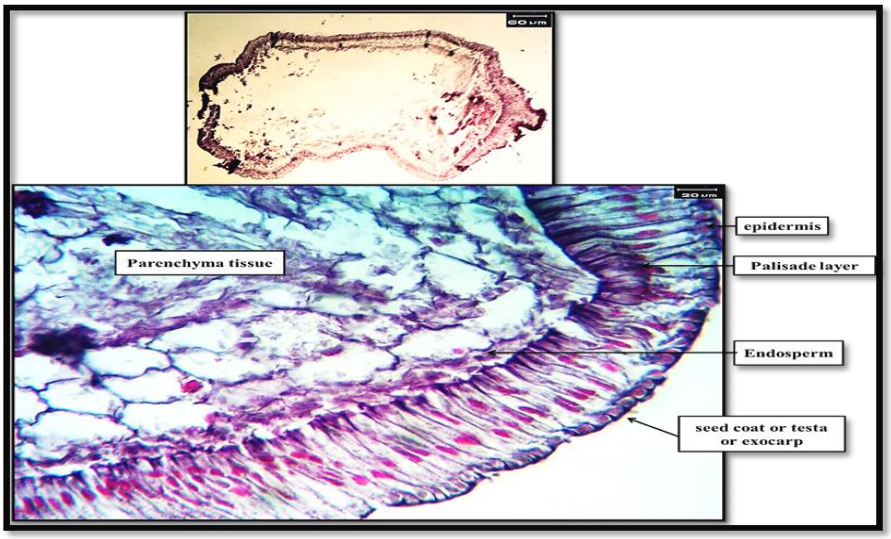

Plate (5): Cross section in the seed of *L. cicera.*

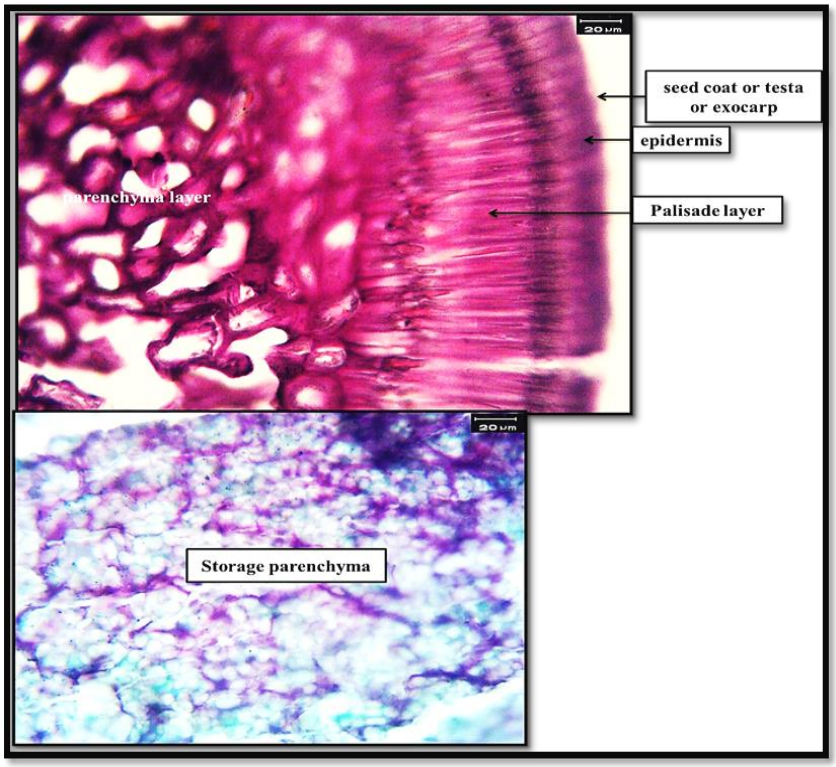

Plate (6): Cross section in the seed of *L. aphaca*.

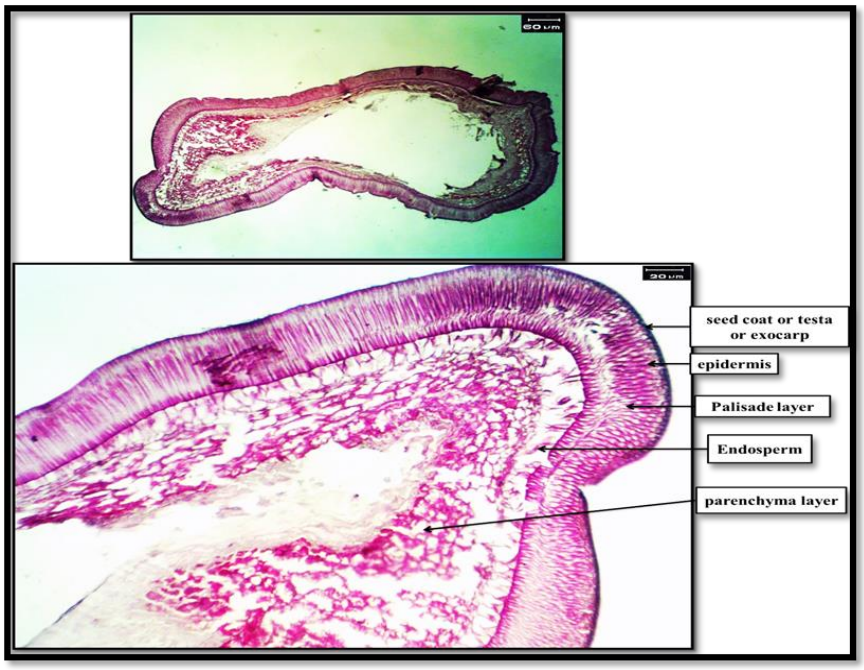

Plate (7): Cross section in the seed of *L. sphaericus*.

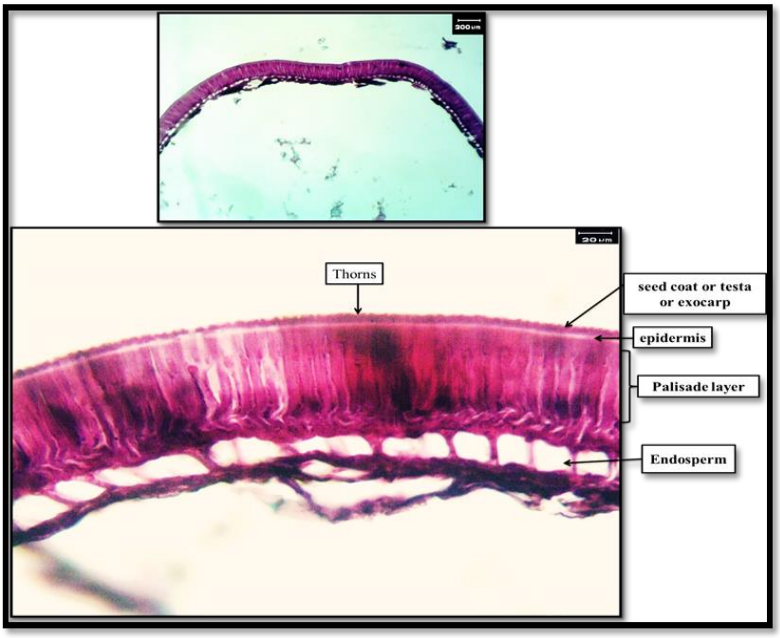

Plate (8): Cross section in the seed of *L. inconspicus*.

Many studies focused on morphology characteristics by using SEM ,such as (Güneş , 2013) in Turkey ,but there is no study interested in *Lathyrus* seed anatomy, in Iraq. This study is considered the first in this field especially for this genus and general for Fabaceae.

DISCUSSION

The species *L. annuus* and *L. cicera* were glossy, and the result pointed out that the presence of wax may be a method for protecting the seeds or may be represented by the nature of secondary metabolism, which involves acumination in the testa. There are variations in the seed coat and specimens between the different species populations. Butler (1988) mentions that the seed characters may directly taxonomy and determined species and have taxonomic values. Numerous researchers like Kislev and Hopf (1935) have emphasized that species cannot be distinguished alone by seed characteristics; nevertheless, when combined with morphological traits, the results obtained in this study are in compliance with the results of Güneş (2013) for some species that were seen in this study (*L. annuus*, *L. aphace*, *L. cicera*).

The result showed that the smooth seeds were not papillate. According to the anatomic features, the testa thickness in *L. annuus* and *L. aphaca* has the highest value. The exocarp (testa) thickness may refer to the nature of the coat's ability of protecting the seed from external factors that cause damage to pathogen (Hall and Stout, 1993). Testa layer thickness and the presence of cuticle require considerable attention as their properties are related to the water-impermeability of hard seeds (Argel and Paton, 1999). Hard-Coatedness is usually caused by the impermeability of the seed coat or some of its layers to water or gases. In *L. sativus* the testa is damaged from sub the sybepidermal in some areas a pit aperture, present at the end of the pit cavity when it junctions with the cavity of the cell ,Wolf *et al*., (1981) and Ma *et al*., (2004) mention that pit irregularities on the seed coat surface seem to be related to its water permeability, and that seems to refer to ability of germination, especially the pit was present in the seed coat of a cultivated plant (*L. sativus*) which is considerd a field crop, and it is used instead of wheat in some regions, such as Nigeria and Somalia.

By cross sections, many unique feathers were distinguished, *L. cicera* species distinguished were by the presence of trichome on the surface of the seed coat that may be considered a way to spread, in addition, there are important characteristics that point out like the osteosclereides in *L. sativus* that differentiate from the subepidermal layer and also consider major cells during testa differentiation. The osteosclerides conjected with a thick wall around the hilum and the continuance of the intercellular space may be related to the seed desiccation and gas exchange (Smýkal *et al.*, 2014). It is important to mention that *L. sativus* had the highest value in parenchyma tissue, which may be duo to his adaptation, and that is one reason to cultivate it. We can also note that the species within the same section of the flora were mentioned almost under the same group, such as:

L. annuus, *L. chloranthus*, *L. sativus*, and *L. cicero*, which belong to the same section (Sativus), (Townsend and Guest, 1974). A little study interested in the anatomy of seeds such

as Jyotindra (1975), it is included the anatomy of hilum for some species belonging to the Fabaceae *Pisum* and *Vicia* in India.

Many studies, such as the study of Güneş (2013) in Turkey, concentrated on the morphology characteristics of *Lathyrus* seeds using SEM, but no studies examined the anatomy of *Lathyrus* seeds; this study in Iraq is thought to be the first in this field, particularly for this genus.

CONCLUSIONS

The morphological characteristics of seeds in the species of the genus could be used as taxonomic evidence and may be reliably combined with the anatomical characteristics of be more useful in the species of the genus. The seeds have a variation in the sculpturing pattern, which provides information to distinguish species of this genus, such as the ornamentation of *L. chloranthus* seed surface, which was distinguished by conical peaks or like needles on the surface of *L. sphaericus* seed. The similar morphology features are considered the evidence for the proximity of the taxa. There are anatomical variations that have a contribution to species delamination such as the epidermis and mesocarp characterstics. The internal structure (anatomy) of the seeds can be used to separate the taxa, the species which belongs to the same section in the flora will be more similar, even in anatomy characteristics, the use of morphology and anatomy side by side will be more beneficial in taxonomic studies. A comprehensive study covering all *Lathyrus* species would be of a beneft to determine the taxa and relationships between the species. The use of molecular studies is necessary in future studies; the Iraqi flora could depend on the SEM result and anatomical characteristics in the taxonomy for separating the species of the genus or genus of the family.

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CONFLECT OF INTEREST STATEMENT

"The author declares no conflicts of interest related to the work in this manuscript".

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دراسة مظهرية وتشريحية مقارنة للبذور بواسطة المجهر الالكتروني الماسح لبعض أنواع الجنس *Lathyrus* L., 1753 (Fabales, Fabaceae) في العراق

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الخلاصة

اجريت دراسة تشريحية مظهرية لسبعة انواع من الجنس *Lathyrus* L.,1753 (العائلة البقولية) في العراق، شملت:

L. annuus L.,1753, *L. aphaca* L.,1753, *L. chloranthus* Boiss.,1859 , *L. cicera* L.,1753 , *L. sativus* L.,1753 , *L. inconspicuus* L.,1753 , *L. sphaericus* Retz.,1753، أذ يوجد في 17 نوع تنتشر في وسط العراق وشماله. دُرست الصفات المظهرية بأستخدام المجهر الماسح الالكتروني لتحديد العلاقة التصنيفية ما بين الانواع، و لحل مشكلة التداخل بين الانواع وتشخيصها وفصلها حسب مراتبها التصنيفية، كانت البذور لامعة في اغلب الانواع عدا النوعين *L.sativus* و *L. sphaericus*، و كان سطح البذور مجعد ومغطى بطبقة من الشمع تتركز على قمة الحليمات، حددت الصفات التشريحية بعمل مقطع عرضي في البذور و درست بعض الصفات مثل الغلاف الخارجي وسمك البرنكيما والنسيج العمادي، سجلت الانواع *L.aphaca* و *L.annuus* اعلى قيمة لسمك الـ exocarp بلغت 5.5 مايكروميتر ، اما النوع *L.sativus* فسجل اقل قيمة بلغت 3.8 مايكروميتر ، وان غلاف البذرة انفصل عن البشرة في بعض المناطق ليكون فتحة النقرة، وقد امكن تقسيم الانواع الى ثلاث مجاميع حسب سمك البشرة الخارجية اذ سجل النوعين *L.sphaericus* و *L.aphaca* اعلى قيم بلغت 46-49 على التوالي، وسجل النوع *L.sphaericus* اعلى قيمة لسمك النسيج العمادي بلغت 61 مايكروميتر، وانفردت بعض الانواع بصفات خاصة مثل وجود النقر في exocarp ، وامتاز كذلك النوع *L.sativus* بوجود تجويف النقرة على طول الطبقة البرنكيمية، و يمكن ان يدل التشابه في الصفات الوراثية على التقارب مابين المراتب التصنيفية وكذلك الحال بالنسبة للتركيب الداخلي (التشريحي) للبذور الذي من الممكن الاستفادة منه في فصل المراتب.