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Anatomical study of vegetative parts of some *Lotus* L. species in Egypt

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Abstract

The anatomical characteristics of ten species belonging to the genus *Lotus* L.; *L. arabicus* L., *L. creticus* L., *L. cytisoides L.*, *L. edulis* L., *L. glaber* Mill. *L. glinoides* Delile, *L. halophilus* Boiss., *L. ornithopodioides* L., *L. polyphyllos* Clarke, and *L. tetragonolobus* L. The subtribe Lotinae are investigated using a light Microscope. The results revealed that anatomical characteristics of the stem (outline, epidermal cell shape, number and size of vascular bundles, crystals and tannin cells in both cortex and pith), leaf (midrib region shape, differentiation of mesophyll cells, and presence of tannin cells and crystals) are of taxonomic significance in differentiation and delimitation between the studied species. A dichotomous artificial key is given based on the studied features.

Keywords: Anatomy; leaf; Lotea; Lotineae; stem

Introduction

The cosmopolitan genus *Lotus* L. belonged to the subtribe Lotinae, tribe Loteae, the largest genus with about 100 and 188 species. It is found in two major centers of diversity; Western North America and the Mediterranean region, which includes parts of Europe, Africa, and Western Asia (Polhill 1981, Gunn 1983, Sokoloff 1998, Kirkbride 1999, Degtjareva *et al*. 2006). The genus *Lotus* ca. 190 species and reduced the tribe Loteae to four genera (Polhill 1981). The genus *Lotus* L. is polymorphic including about 150 species native to Europe, Asia, Africa, Australia, and some islands of the Atlantic Ocean, Pacific Ocean, and Socotra archipelago in the Indian Ocean.

There are over 200 species of the intricate genus *Lotus* in existence worldwide. There are two regions with great species diversity: the Mediterranean region, which includes Macaronesia (approximately 135 species), and western North America (about 50 species) (Allan *et al*. 2003). Egypt has recorded twenty species of *Lotus* (Täckholm 1974, Boulos 1995, El Hadidy 2003). While Boulos (1999, 2009) found eighteen species, El-Hadidi & Fayed (1994/1995) documented twenty-two. Three subgenera Pedasia, Lotus, and Tetragonolobus and four sections—Krokeria, Lotea, Lotus, and Erythrolotus were adopted by El Hadidy (2003, 2004) based on floral characteristics (style and stigma), fruit characteristics (pod and seed), vegetative characteristics (basal leaflets), and geographic distribution.

Barykina and Krammina (2006) differentiated between three *Lotus* species, viz. *L. corniculatus*, *L. japonicus* and *L. miyakojimae* Kramina anatomically in terms of thick cork and reserve starch in the root. In addition, the number of main conductive bundles along the whole shoot,

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the lack of additional bundles, thick leaf blades and surface epidermal cells, many idioblasts with anthocyanins and phlobaphenes in the primary cortex of the stem and the leaf tissues are used.

Maroso et al. (2009) used the anatomical structures; pith cell integrity, starch content in the parenchyma root cell, and underground stem cortex to differentiate between *L. corniculatus* and *L. uliginosus*.

The anatomical structures are useful in taxonomic characterization of plants. At several systematic levels, the distribution and arrangement of tissues and cells, including vascular bundles, sclerenchyma, and other morphological traits, have been documented and used to delimit taxa. (Agbagwa & Ndukwu 2004, Ajuru, 2012).

Trang *et al*. (2019), studied the anatomy of six *Lotus* varieties collected at different planting areas in Vietnam; their findings show that although the six varieties are cultivated under the same natural conditions, they are highly diversified in terms of the internal anatomical features and to reveal their important diagnostic characters.

The current study uses a light microscope to examine the relationships among *Lotus species* to obtain more thorough anatomical descriptions and diagnostic traits since there hasn't been a prior comparative anatomical investigation.

Materials and Methods

For the investigation, fresh samples of the stems and leaves of ten species of *Lotus* were chosen (Table 1**).** The examination was made at the fruiting stage and in comparable positions of each plant. Stem samples were taken from 4th internodes from the apex about 2–3 cm. Fresh materials were fixed in FAA (Formalin–glacial acetic acid–70% ethyl alcohol, 5:5:90 V/V). After 24-hour fixation, the specimens were transferred into an Ethyl alcohol series and embedded in paraffin wax. The specimens were sectioned by a rotary microtome at 10–15 µm; sections were dehydrated in alcohol–xylol series and stained by safranin and light green according to Sass 1961. The anatomical characteristics of stem and leaves were examined using a Zeiss research light microscope and photographed with a digital camera.

A planimeter was used to measure the percentage of each tissue to the total section area. Terminology followed by Abd El–Rahman et al. (1976), Pandey (1982), and Abd El–Gawad et al. (1989).

Table 1. Plant names, collection details, and sources of the studied *Lotus* L. species.

Taxa	Sections Degtjareva & al. (2006)	Source of seeds	Localities	Geographical Coordinates	Elevation (m) above sea level	Date of collection
L. arabicus L.	Heinekenia	XDL 90-00 26D	United States of America	-	-	-
L. creticus L.	Pedrosia	XDL 92-0078	Palestine	-	-	-
L. cytisoides L.	Lotea	-	Wadi Halq El-Dabe- Marsa Matrouh	31°27.466ˈN 26° 47.461ˈE	3	27/3/2014
L. edulis L.	Krokeria	-	Wadi Umm El-Rakham, Marsa Matrouh	31°23.978ˈN 27° 01.149ˈE	6	22/3/2019
L. glaber Mill.	Lotus	-	Alexandria, Marsa Matrouh Road	30°56.848ˈN 29° 30.923ˈE	9	15/3/2019
L. glinoides Del.	Chamaelotus	-	Wadi Hagul	29°51.295ˈN 32°15.830ˈE 29°51.290ˈN 32° 15.879ˈ E	162	3/4/2015 1/4/2016 17/3/2017 10/3/2018 8/3/2019
L. halophilus Boiss.	Lotea	-	El-Mathani El-Bahria - Marsa Matrouh	31°27.963ˈN 26° 45.287ˈE	8	23/3/2017
L. ornithopodioides L.	Lotea	-	Burg El-Arab	30°55.306ˈN 29° 31.560ˈ E	11	25/3/2016
L. polyphyllos Clarke	Lotea	-	Agiba beach	31°24.858ˈN 27° 00.339ˈE	6	8/4/2016
L. tetragonolobus L.	-	-	El-Mathani El-Bahria, Marsa Matrouh	31°27.676ˈN 26° 41.575ˈE	13	23/3/2018
	-	-	El-Mathani El-Bahria, Marsa Matrouh	31°27.963ˈN 26° 45°.287ˈE	8	23/3/2017
	-	-	City of Scientific Research Station- Burg El-Arab	30°53.492ˈN 29° 32°.717ˈE	21	31/3/2017

Results

Stem anatomy

The outline of the stem is circular- angular with 4-6 ridges. Epidermal cells show a wide variety of differentiation; tangentially elongated-tubular, tangentially elongated- isodiametric, isodiametric, radially elongated- isodiametric, radially elongated, with a very thin, wavy cuticle layer. Cortex with outer 3–7 layers of tangentially elongated-tubular, tangentially elongated- isodiametric, tangentially elongated parenchyma, sand crystals and tannin cells present in some species.3–7 layers of pericyclic fiber patches are present outside vascular bundles, followed by 3-6 layers of phloem. Xylem with 2–7 vessels, mainly in radial groups of 1–7 arches. Pith is formed of isodiametric-polygonal-radially elongated parenchymatic cells, sand crystals are absent in most studied species; and tannins are present in the cortex of all species while present in pith in *L. arabicus* L., *L. glaber* Mill., *L. glinoides* Del., *L. polyphyllos* Clarke and *L. tetragonolobus* L. (Figs 1-4, Table 2).

The following is a summary of the notable variations found in the anatomical investigations of their stems; outline of stem is circular in *L. arabicus* L., *L. edulis* L., *L. glinoides* Delile, *L. halophilus* Boiss., *L. ornithopodioides* L., *L. polyphyllos* Clarke and *L. tetragonolobus* L., Angular with 4–ridges in *L. cytisoides L.*, *L. glaber* Mill. and with 6–ridges in *L. creticus* L.

Epidermal cells are radially elongated in *L. creticus* and *L. polyphyllos*, isodiametric in *L. cytisoides*, tangentially elongated-tubular in *L. arabicus* and tangentially elongated- isodiametric in *L. edulis, L. ornithopodioides* and *L. tetragonolobus*. Cortex consists of 3–5 (7) layers parenchyma in all studied *Lotus* species.

Number of vascular bundles: 8–11 bundles in *L. arabicus, L. glaber, L. ornithopodioides, L. polyphyllos* and *L. tetragonolobus,* 12–13 bundles in *L. cytisoides, L. edulis, L. halophilus* and 14–16 bundles in *L. creticus, L. glinoides.*

The examined species are categorized based on the presence of solitary crystals in their stems as follows: crystals are present in both cortex and pith only in *L. tetragonolobus* and absent in *L. arabicus, L. creticus, L. cytisoides, L. edulis, L. glaber, L. glinoides, L. halophilus, L. ornithopodioides* and *L. polyphyllos.* In terms of tannin cell presence; all the studied species have tannin in cortex while *L. arabicus, L. glaber, L. glinoides, L. polyphyllos* and *L. tetragonolobus* have tannin in pith and absent in *L. creticus, L. cytisoides, L. edulis, L. halophilus* and *L. ornithopodioides.*

Leaf Anatomy

Leaf is bifacial, dorsiventral with u-v shaped midrib region. In midrib region; epidermal tissue represented by one layer covered with wavy cuticle that varies in shape from isodiametric–radially–tangentially elongated, followed by 2–5 parenchyma layers with isodiametric–tangentially, to radially elongated cells. The main closed vascular bundle represented by 2–5 xylem arches, each arch with 2–4

vessels and phloem in 2–4 layers. In the wings region; mesophyll consists of one type of cell, distinguished into palisade and spongy tissue, at the later type, palisade tissue is recorded as present on both surfaces of leaves or observed only towards the upper side. Crystals and tannin cells present or absent in midrib region and wings (Figs 4-5, Table 3).

The main distinctions among the examined species are enumerated as follows: v– shape midrib region present in *L. creticus, L. cytisoides, L. glaber, L. glinoides* and *L. polyphyllos* and u–shape in *L. arabicus, L. edulis, L. halophilus, L. ornithopodioides* and *L. tetragonolobus, c*rystals are present in midrib region only in *L. cytisoides, L. halophilus* and absent in *L. arabicus, L. creticus, L. edulis, L. glaber, L. glinoides, L. halophilus, L. ornithopodioides* and *L. tetragonolobus.*

In wings region; Mesophyll consists of one type of cells in *L. creticus, L. glaber, L. glinoides* and *L. tetragonolobus;* 5-6 layers in *L. glaber* and 7-10 layers in *L. creticus, L. glinoides* and *L. tetragonolobus.* While distinguished into palisade and spongy tissue in *L. arabicus, L. cytisoides, L. edulis, L. halophilus, L. ornithopodioides* and *L. polyphyllos*. Tanin cells absent in *L. arabicus, L. glaber* and *L. creticus, L. cytisoides, L. edulis, L. glinoides, L. halophilus, L. ornithopodioides, L. polyphyllos* and *L. tetragonolobus* (Figs 4- 5, Table 3).

Palisade tissue recorded as present on both surfaces in certain species; *L. arabicus, L. cytisoides, L. halophilus* and *L. polyphyllos* while observed only towards the upper side of leaves in *L. edulis* and *L. ornithopodioides*.

Table 2. Anatomical characters of stem in the studied *Lotus* species

Species	Outline	Number of ridges	Diameter (µm)	Cuticle thickness (µm)	Epidermal cell		Cortex		
					Shape	Width (µm)	Parenchyma		
							Number of layers	Width (µm)	Cell shape
L. arabicus	Circular	-	950-975	Up to2.5	Tangentially elongated-tubular	15-27.5	3-4	42.5-75	Tangentially elongated-tubular
L. creticus	Angular	6	1025-1125	5-7.5	Radially elongated	15-17.5	4-5	50-62.5	Tangentially elongated- isodiametric
L. cytisoides	Angular	4	1225-1275	5	Isodiametric	12.5-15	5-6	45-75	Tangentially elongated- isodiametric
L. edulis	Circular	-	1075-1125	Up to 2.5	Tangentially elongated-isodiametric	15-22.5	5-6	55-70	Tangentially elongated
L. glaber	Angular	4	925-950	5	Radially elongated-isodiametric	15-17.5	4-5	45-67.5	Tangentially elongated- isodiametric
L. glinoides	Circular	-	1150-1175	2.5	Radially elongated	12.5-25	4-5	40-62.5	Tangentially elongated- isodiametric
L. halophilus	Circular	-	850-900	Up to 5	Radially elongated-isodiametric	10-15	4-5	37.5-57.5	Tangentially elongated- isodiametric
L. ornithopodioides	Circular	-	875-1000	Up to 2.5	Tangentially elongated-isodiametric	17.5-27.5	4-5	62.5-82.5	Tangentially elongated- isodiametric
L. polyphyllos	Circular	-	1050-1075	Up to 5	Radially elongated	15-17.5	4-5	70-100	Tangentially elongated
L. tetragonolobus	Circular	-	1100-1175	2.5-5	Tangentially elongated-isodiametric	20-25	4-7	92.5-125	Tangentially elongated- isodiametric

Table 2. Continued

Species	Vascular bundles		Pericyclic fiber		Phloem		Cambium		Xylem		
	Number	Width (µm)	Number of layers	Width (µm)	Number of layers	Width (µm)	Number of layers	Width (µm)	Number of arches	Number of layers vessels	Width (µm)
L. arabicus	11	130-160	3-4	25-35	5-6	32.5-40	2	10	3-6	4-5	60-137.5
L. creticus	15-16	150-200	4-5	25-50	4-5	30-45	2	7.5-10	2-4	2-4	62.5-95v
L. cytisoides	12-13	140-240	6-7	40-67.5	5-6	37.5-55	2	7.5-10	3-5	3-5	65-137.5
L. edulis	12-13	120-180	4-5	37.5-47.5	4-5	17.5-20	2	10	3-4	3-4	67.5-87.5
L. glaber	10	160-260	4-5	32.5-82.5	4-5	25-35	2	10	2-5	2-3	50-95
L. glinoides	14-15	160-220	4-5	30-45	4-5	27.5-35	2	7.5-10	1-6	3-5	95-137.5
L. halophilus	12	140-200	3-4	30-37.5	4-5	30-35	2	10	2-5	2-4	62.5-105
L. ornithopodioides	10-11	120-220	3-5	35-37.5	4-6	17.5-25	2	10	1-5	3-5	62.5-100
L. polyphyllos	11-12	130-240	4-5	45-62.5	3-4	30-35	2	7.5-10	3-5	3-7	80-130
L. tetragonolobus	10-12	190-205	6-7	62.5-75	5-6	27.5-40	2	10	2-7	3-7	40-75

Table 2. Continued

Species	Stem character					
	Pith		Crystals	Tannin cells		
	Cell shape	Diameter (µm)	In cortex	In pith	In cortex	In pith
L. arabicus	Isodiametric-polygonal	520-550	Absent	Absent	18-20	4-10
L. creticus	Isodiametric	450-500	Absent	Absent	36-38	Absent
L. cytisoides	Isodiametric-cylindrical	575-625	Absent	Absent	40-48	Absent
L. edulis	Radially elongated-polygonal	530-550	Absent	Absent	27-28	Absent
L. glaber	Isodiametric-irregular	430-450	Absent	Absent	22-26	6-8
L. glinoides	Isodiametric-irregular	560-600	Absent	Absent	28-32	17-21
L. halophilus	Isodiametric-cylindrical	380-420	Absent	Absent	35-39	Absent
L. ornithopodioides	Isodiametric-cylindrical	310-400	Absent	Absent	22-23	Absent
L. polyphyllos	Radially elongated- isodiametric – polygonal	330-350	Absent	Absent	41-45	4-7
L. tetragonolobus	Isodiametric-polygonal	500-525	Solitary	Solitary	10-14	3-4

Table 3. Anatomical characters of leaf in the studied Lotus species. Abbreviations: up. ep.: Upper epidermis, lo. ep.: Lower epidermis, Pal.: palisade tissue, spo.: spongy tissue.

Species	Leaf midrib character									
	Shape	Midrib thickness (µm)	Cuticle thickness (µm)	Epidermal cells		Xylem			Phloem	
				Thickness (µm)	Shape	Number of arches	Thickness (µm)	Number of vessels in arch	Number of layers	Thickness (µm)
L. arabicus	U	500-550	2.5	25-37.5 up.ep 15-25 lo.ep.	Isodiametric-tangentially elongated	4	62.5-75	2-4	3-4	20-30
L. creticus	V	280-300	2.5	20-30 up.ep 22.5-27.5 lo.ep.	Radially elongated	3-4	35-42.5	3-4	3	10-12.5
L. cytisoides	V	400-420	2.5	35-47.5 up.ep 25-42.5 lo.ep.	Radially-tangentially elongated-polygonal	2-3	55-62.5	2-3	2-3	20-25
L. edulis	U	300-330	2.5-5	20-30 up.ep 15-30 lo.ep.	Isodiametric-tangentially elongated	3	45-55	2-3	4	17.5-20
L. glaber	V	350-380	2.5-5	30-42.5 up.ep 27.5-50 lo.ep.	Isodiametric-tangentially elongated	3-4	42.5-50	2-3	4	15-25
L. glinoides	V	400-500	2.5	17.5-25 up.ep 25-42.5 lo.ep.	Isodiametric-tangentially-radially elongated	3	55-70	3-4	3	15-20
L. halophilus	U	380-400	2.5-5	15-25 up.ep. 17.5-30 lo.ep.	Isodiametric-tangentially elongated	2-3	42.5-60	2-4	4	15-20

L. ornithopodioides	U	350-400	2.5	25-30 up.ep. 12.5-32.5 lo.ep.	Isodiametric-tangentially-radially elongated	3-4	62.5-67.5	3-4	4	17.5-20
L. polyphyllos	V	580-620	2.5	27.5-37.5 up.ep. 22.5-30 lo.ep.	Radially elongated	4	57.5-62.5	2-4	2	12.5-15
L. tetragonolobus	U	320-400	2.5	25-35 up.ep. 20-27.5 lo.ep.	Isodiametric-radially-tangentially elongated	3-4	40-42.5	2-4	3	15-17.5

Table 3. Continued

Species	**Leaf midrib character**						
	Number of parenchyma layers	**Parenchyma**		**Number of fiber layers**	**Fiber thickness (µm)**	**Tannin cells**	
		Thickness (µm)	**Cell shape**			**Upper vascular bundle**	**Lower vascular bundle**
L. arabicus	2-3 up.ep. 2-3 lo.ep.	100-125 up.ep. 80-112.5 lo.ep.	Isodiametric-radially elongated	2	15-20	1-2	Absent
L. creticus	2-3 up.ep. 2-3 lo.ep.	25-57.5 up.ep. 32.5-37.5 lo.ep.	Radially elongated	2	15-17.5	1-3	Absent
L. cytisoides	2 up.ep. 2-3 lo.ep.	70-90 up.ep. 37.5-80 lo.ep.	Isodiametric	2	25-30	Absent	Absent

L. edulis	2 up.ep. 2 lo.ep.	25-45 up.ep. 50-70 lo.ep.	Isodiametric-tangentially elongated	2	15-22.5	Absent	Absent
L. glaber	2-3 up.ep. 2 lo.ep.	50-55 up.ep. 42.5-57.5 lo.ep.	Radially elongated-isodiametric	1-2	12.5-25	1-2	Absent
L. glinoides	4 up.ep. 2-3 lo.ep.	95-112.5 up.ep. 75-105 lo.ep.	Radially elongated	1-2	12.5-20	2-3	12-15
L. halophilus	3 up.ep. 2-3 lo.ep.	87.5-100 up.ep. 37.5-42.5 lo.ep.	Isodiametric	1-2	12.5-17.5	Absent	Absent
L. ornithopodioides	2 up.ep. 2 lo.ep.	67.5-75 up.ep. 62.5-65 lo.ep.	Radially elongated-isodiametric	2	22.5-25	1	1
L. polyphyllos	3-5 up.ep. 6-7 lo.ep.	150-180 up.ep. 180-220 lo.ep.	Tangentially elongated	1	12.5-17.5	2	Absent
L. tetragonolobus	3-4 up.ep. 4-5 lo.ep.	75-87.5 up.ep. 105-125 lo.ep.	Isodiametric	2	15-17.5	1	2

Table 3. Continued

Species	Mesophyll		Palisade layer		Spongy layer		Tannin cells
	Number of rows	Thickness (µm)	Number of rows	Thickness (µm)	Number of rows	Thickness (µm)	
L. arabicus	-	-	1-2 up.ep. 2-3 lo.ep.	87.5-125 up.ep. 50-87.5 lo.ep.	2-3	72.5-87.5	Absent
L. creticus	7-9	150-187.5	-	-	-	-	Many
L. cytisoides	-	-	2 up.ep. 2 lo.ep.	87.5-92.5	3	80-85	7-11
L. edulis	-	-	2	65-70	3	65-67.5	10-15
L. glaber	5-6	105-150	-	-	-	-	Absent
L. glinoides	7-10	250-310	-	-	-	-	Many
L. halophilus	-	-	2 up.ep. 3 lo.ep.	57.5-62.5 up.ep. 100-112.5 lo.ep.	2-3	45-55	10-12
L. ornithopodioides	-	-	2	55-67.5	3-4	85-87.5	2-6 in pal
L. polyphyllos	-	-	3 up.ep. 3 lo.ep.	80-125	2-3	62.5-75.5	Many in spo
L. tetragonolobus	7-8	150-162.5	-	-	-	-	4-15

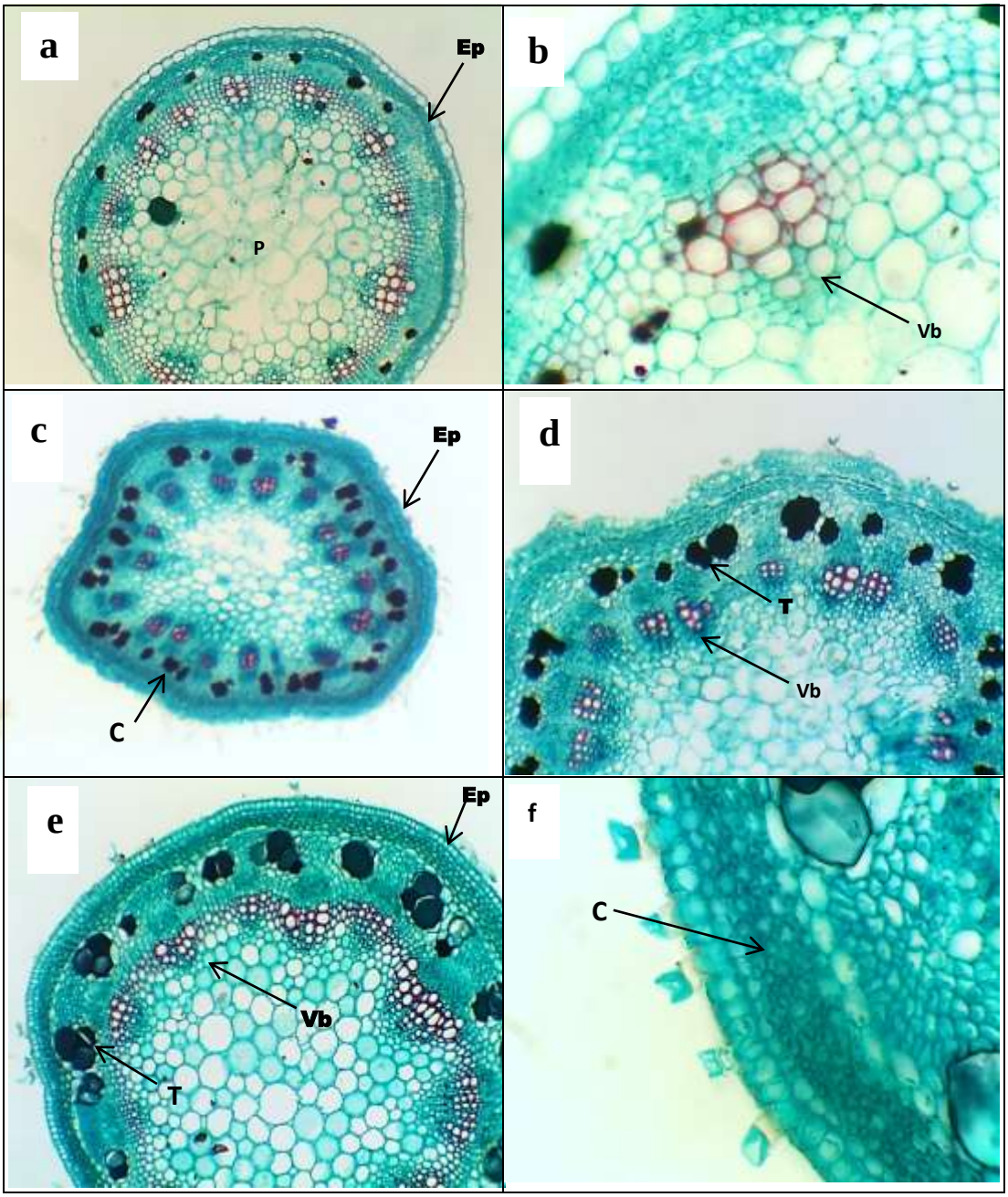

Figure 1. Anatomical Characters of stem of *Lotus* species a, b. L. *arabicus*; c, d. L. *criticus*; e, f. *L. cytisoides, a*. whole stem, b. stem sectors. (a x50, b x100). Abbreviations: Ep. Epidermis, C. cortex, Vb. vascular bundles, P. Pith, T. Tannin cell.

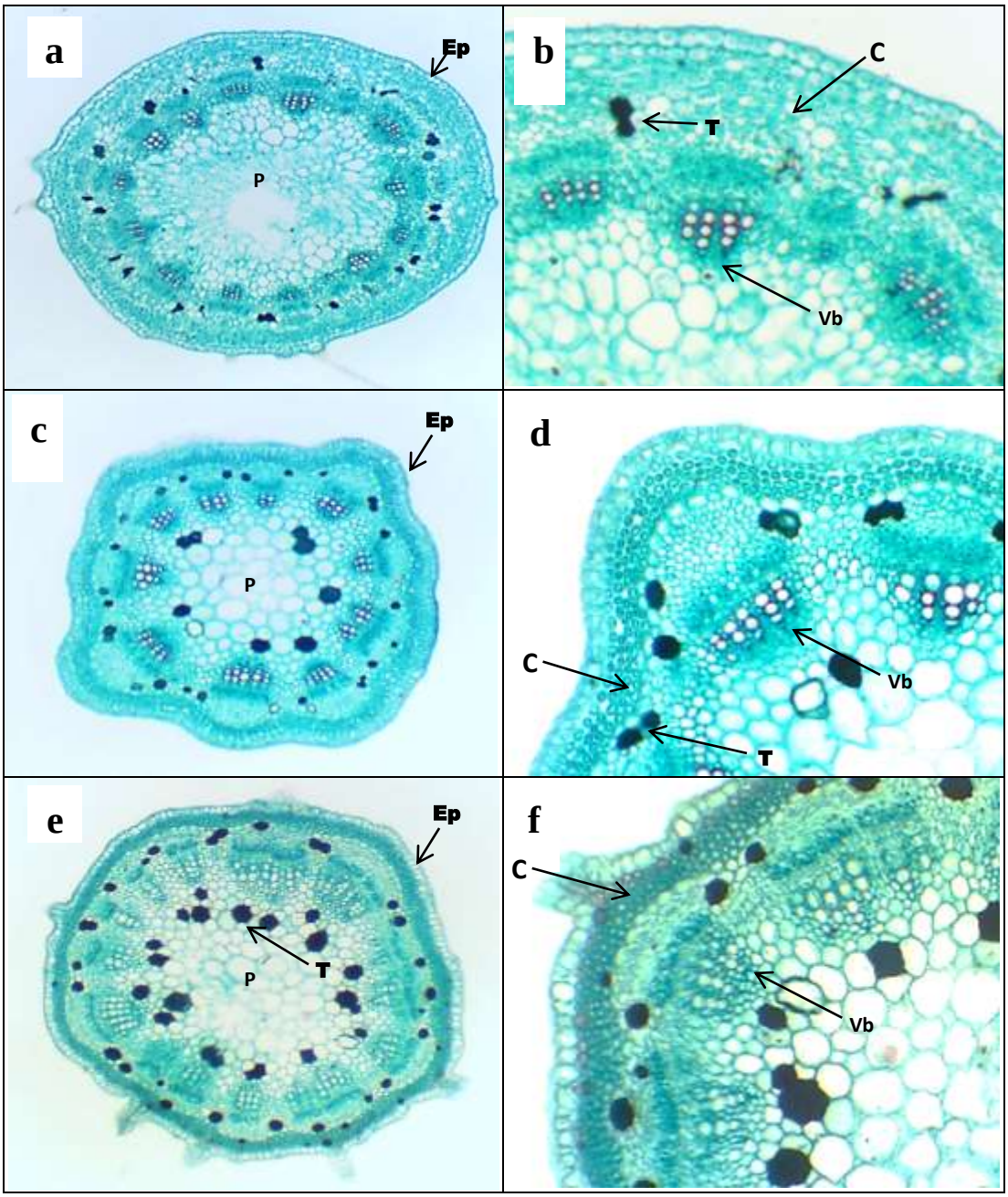

Figure 2. Anatomical Characters of stem of *Lotus* species a, b. L. *edulis* c, d. *L. glaber*, e, f. *L. glinoides; a*. whole stem, b. stem sectors. (a x50, b x100). Abbreviations: Ep. Epidermis, C. cortex, Vb. vascular bundles, P. Pith, T. Tannin cell.

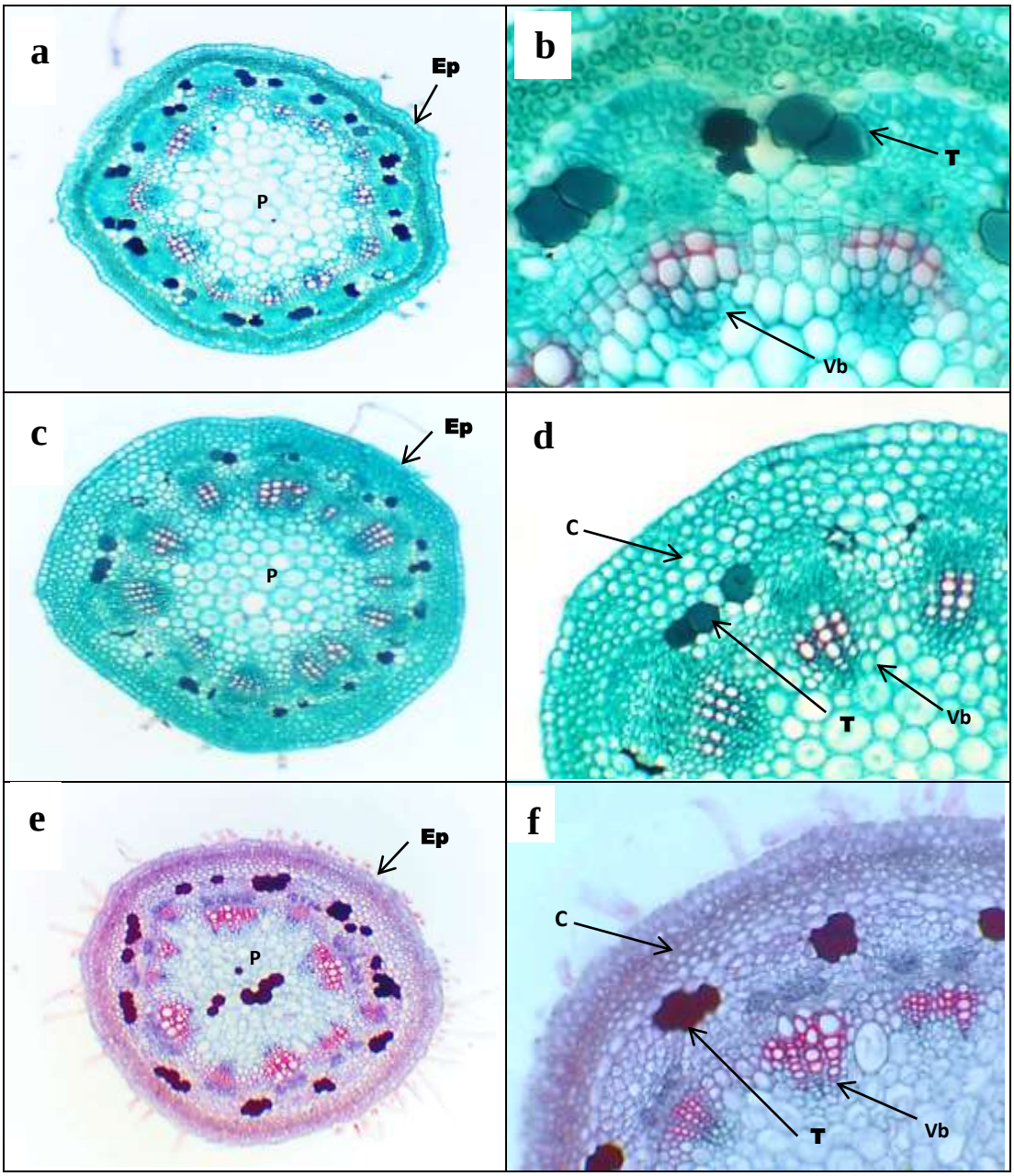

Figure 3. Anatomical Characters of stem of *Lotus* species a, b; *L. halophilus*, c, d. *L. ornithopodioides*, e, f. *L. polyphyllus.* a. whole stem, b. stem sectors. Stem anatomy (a x50, b x100). Abbreviations: Ep. Epidermis, C. cortex, Vb. vascular bundles, P. Pith, T. Tannin cell.

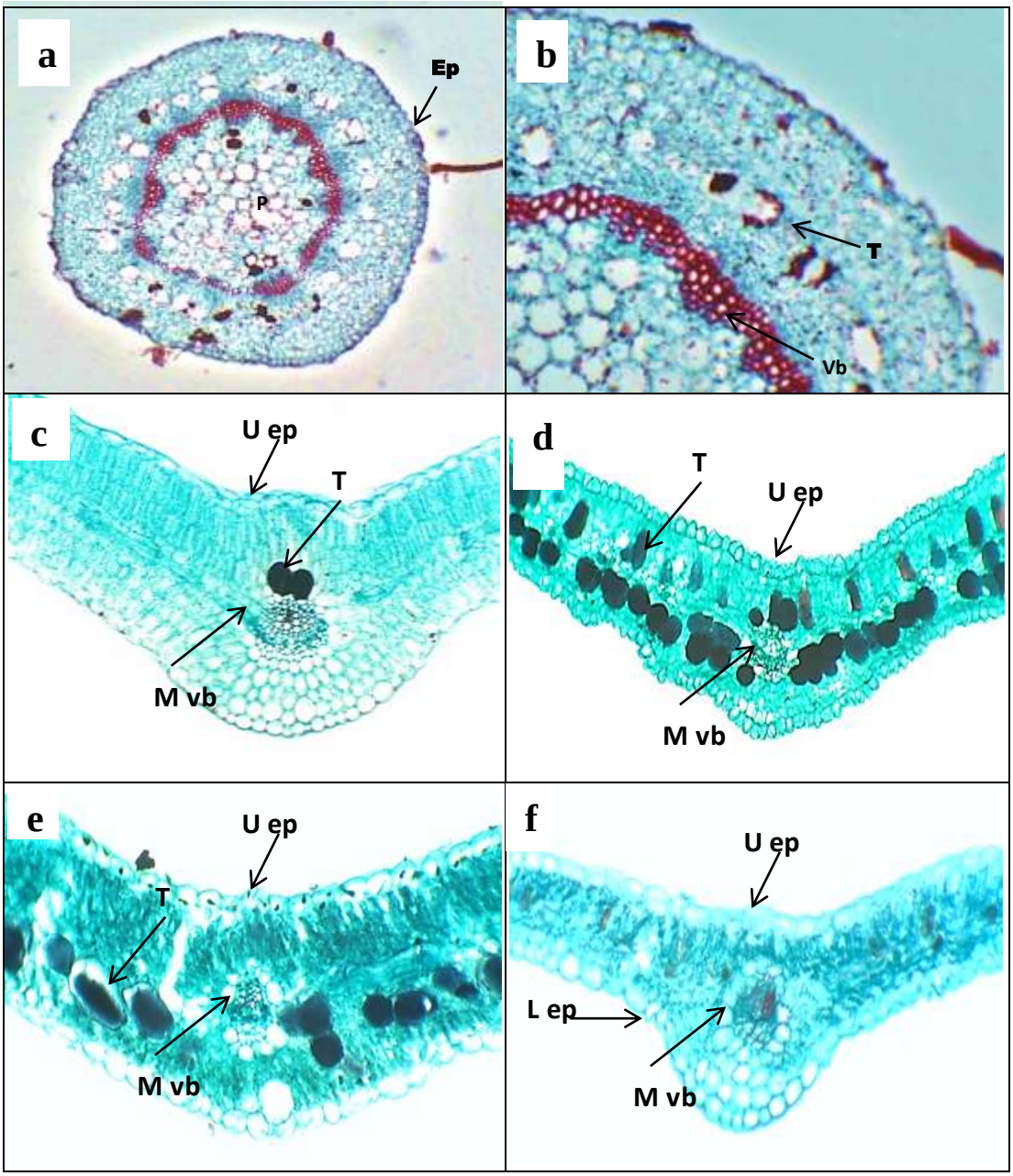

Figure 4. Anatomical Characters of stem and leaf of *Lotus* species a, b. *L. tetragonolobus*; c, *L. arabicus;* d. *L. creticus*; e, L. *cytisoides*, f, L. *edulis*. a. whole stem, b. stem sectors, c-f. leaves. Stem anatomy (a, c-f x50, b x100). Abbreviations: Ep. Epidermis, C. cortex, Vb. vascular bundles, P. Pith, T. Tannin cell.

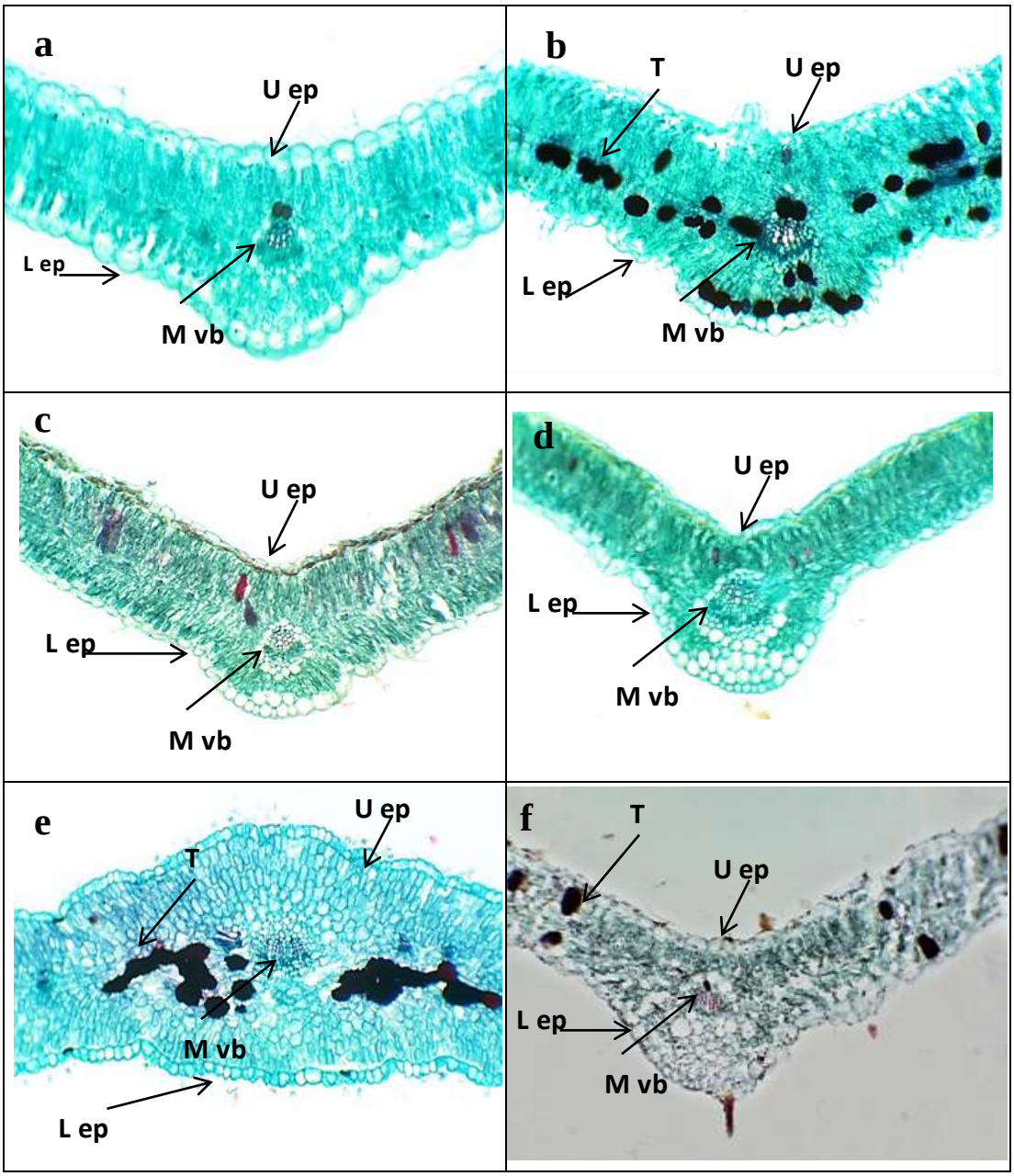

Figure 5. Anatomical Characters of leaves of *Lotus* species. A. *L. glaber*, b. *L. glinoides*, c. *L. halophilus*, d. *L. ornithopodioides, e. L. polyphyllus*; f. *L. tetragonolobus* (x50). Abbreviations: U ep. Upper epidermis, L ep. lower epidermis, M vb. Main vascular bundles, T. Tannin cell.

Discussion

Some prior studies have revealed the anatomical characteristics of the subfamily Papilionoideae and their significance for the taxonomy classification (Metcalfe & Chalk 1950, Krisshnamurthy & Kannabiram 1970, Kannabiram &Krishnamurthy 1974, Kothari & Shah 1975, Lackey 1978, Crow *et al.* 1997, Teixeira & Gabrielli 2006, Mbagwu & Edeoga 2008, Basconsuelo *et al.* 2011, Celep *et al.* 2011, Aguoru & Okoli 2012, Fortuna-Perez *et al.* 2012, Kahraman *et al.* 2014, Devecchi *et al.* 2015, Geetha *et al.* 2016, Trang *et al.* 2019, Aqlan *et al.* 2022& Ramanantsialonina *et al.* 2024).

In the Papilionoideae, the crystals, which are which are primarily solitary but differ widely in size and shape, sometimes have a unique appearance and distribution, especially in the leaf epidermis (Metcalfe & Chalk 1950).

Fabaceae leaves are usually dorsiventral and less frequently isobilateral, variable in structure due to the wide range of leaf types (Metcalfe and Chalk, 1950).

Howard (1979) and Khatijah *et al.* (1992) mentioned that many anatomical characteristics reflect the taxonomic relationship between species. Moreover, anatomical data are used in taxonomic and phylogenetic studies, Illoh & Inyang (1998).

The studied taxa displayed remarkable differences in the anatomical investigations in both stem and leaflet; outline of stem cross-section, number of parenchyma layers, number and size of vascular bundles, the presence of solitary crystals in the cortex and pith, the presence of tannin cells, midrib region shape, and mesophyll differentiation tissue in the leaf significantly differentiate *Lotus* species, these findings are consistent with those of Barykina & Krammina (2006) and Trang et al. (2019).

Conclusion

Significant differences between Egyptian *Lotus* species were observed in this study, which is the first to examine the anatomical traits of vegetative organs used in the differentiation and development of an artificial key for the examined *Lotus* species.

Artificial key to the studied *Lotus* species

1a- Solitary crystals present in cortex and pith……..….***L. tetragonolobus***
1b- Solitary crystals absent in cortex and pith ……..……2
2a-Tannin cells present in pith…….……..……...*3*
2b- Tannin cells absent in pith…….....……..…….6
3a- Leaf midrib region u-shaped……...……***L. arabicus***
3b- Leaf midrib region v-shaped……...……..……..4
4a- Vascular bundles in stem 14-15…… ***L. glinoides***
4b- Vascular bundles in stem 10-12……..……5

5a-Leaf epidermal cells isodiametric-tangentially elongated, stem outline angular ……..…. ***L. glaber***
5b-Leaf epidermal cells radially elongated, stem outline circular ... ***L. polyphyllos***
6a- Mesophyll one type of cells……...…….. ***L. creticus***
6b- Mesophyll two types of cells……..…….7
7a- Palisade tissue present on both leaf surfaces…….…..8
7b- Palisade tissue present on upper leaf surface only…..…...……9
8a-V-shaped midrib region…… ***L. cytisoides***
8b- U-shaped midrib region…….….***L. halophilus***
9a- Tannin cells absent in leaf vascular bundles, 27-28 tannin cells in stem …… ……***L. edulis***
9b- Tannin cells present in leaf vascular bundles, 22-25 tannin cells in stem..... ..... ***L. ornithopodioides***

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