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ORIGINAL STUDY

A Comparative Anatomical Study of Stem and Leaves of the Genus *Trigonella* L. Species (Fabaceae) in North of Iraq

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Abstract

Anatomical evaluation of 11 *Trigonella* L. species was carried out after preserving the sample parts in FAA solution, and the sample tissues were sectioned by the paraffin method. The results revealed significant anatomical feature differences among the stem and leaf parts in the studied species. Although, prismatic crystals and tannins were present in all the species, the shape of the stem, petiole, midrib, margin outlines, and vascular bundles arrangements were anatomically varied, as well as the presence of hairs, that were unicellular glandular or non-glandular among the species. The fiber and collenchyma tissues exist in stems, petioles, and midribs in all studied species. Stem outline shapes differed between taxa, however, *T. caelesyriaca* differed from the other species in the vascular bundles which were loosely arranged, and in *T. strangulate* were three large vascular bundles interruptedly arranged in a ring. In spite of the variations among the petiole outlines no accessory vascular bundles were observed in *T. aurantiaca*. The leaflet midribs showed different outlines, the accessory vascular bundles were observed only in *T. monantha* var. *monantha* and *T. aurantiaca*. As for the differences observed in the margins of the leaflets were rounded upwards in most taxa, nevertheless, the margin is rounded straight in *T. spicata*, *T. stellate*, and *Trigonella foenum-graecum*; rounded downward in *T. monspeliaca*, *T. caelesyriaca*, and *T. aurantiaca*; where pointed upwards in *T. monantha* var. *monantha* and *T. filipes*.

Keywords: Anatomy, Characteristics, Cross sections, Fabaceae, Paraffin blocks, Petiole, *Trigonella*

1. Introduction

The Fabaceae is largest family of angiosperms after Asteraceae and Orchidaceae in the cosmopolitan perimeter especially in equatorial, subtropical regions, also phlegmatic mountainous regions in Europe, Asia and North America plants [1−3]. This is generally renowned as the legume, pea, or bean family; implicated 650 to 770 genera with 18,000−19,500 species around the world, they are famed family and have an economic usefulness of flowering plants [4,5]. In Brazil, 2848 species are familiar; 1539 species are endemic under 222 genera [6]. Enormous species of the Fabaceae family are economically important, it is used in fields of food and fuel, as a source of organic mineral ores [7,8], due to the high nutritional value of bean seeds, it regarded as a source of food for various poor countries worldwide [9]. As well as the species are useful as a concerned resource of atmospheric nitrogen when the rate is fixed in the cover of the earth [10,11].

The Fabaceae or Leguminosae, are encompass trees, shrubs and herbaceous plants perennials or annuals, which are easily differentiate by their fruits as legume and their complex stipulated leaves. The anatomical features of the genera *Securigera* DC., *Sophora* L. and *Taverniera* DC. stems are of interest

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classification is sometimes equal to morphological traits that are helpful to isolate species [12]. A comparative anatomical was involved in order to identify the structure of the vegetative and reproductive organs and to evaluate the taxonomic significance of qualitative characters for set apart among the taxa. The combination traits of chosen quantitative anatomical particulars of the petiole, stipules, stem, peduncle and calyx; that the previously not studied in detail were significant in delimiting sections of the genus *Trifolium* [13]. Aldabbagh [14] used only the petiole characteristics and constructed an anatomical key to separate the taxa of the genus *Trifolium*.

Trigonella L. comprises approximately 135 species that grow naturally in Iraq, they are distributed in dry areas around the Mediterranean, Western Asia, and Europe [15,16]. A few researchers have studied the taxonomical study of the genus *Trigonella* on the bases of pod shape, thickness, and the number of seeds per pod. The genus was classified according to flower, legumes, and seed surface aspects into two subgenus and eight sections [17,18]. Taia [19], observed the considerable variation in seed shape and seed coat ornamentation within *Trigonella*. (Ceter [20] morphologically studied seeds of 37 taxa of *Trigonella* they divided the studied taxa on the basis of seeds into twelve sections based on seed traits and described seven characteristics detecting the existence of great variations. *Trigonella* is recognized, not only as a break-crop for cereal trends but also as a medicinal plant, source of diosgenin and other elements such as protein source, mucilage, in addition to the culinary uses for flavoring [21]. Turki et al. [22], studied only the morphology of seed traits in 19 taxa of *Trigonella* using light and an electronic microscope, they constructed a taxonomic key to classify studied species based on the shape and surface aspects of the seeds of the studied species.

The aim of this study is the comparative stem and leaf anatomy among species of the genus *Trigonella* such as: (*T. spruneriana* Boiss. Diagn., *T. spicata* Sm. In Sibth. & Sm., *T. monantha* var. *monantha* C. A. Mey., Ver., *T. monspeliaca* L., *T. caelesyriaca* Boiss. Diagn., *T. aurantiaca* Boiss., Diagn., *T. filipes* Boiss. Diagn., *T. strangulate* Boiss. Diagn., *T. stellate* Forssk., *T foenum-graecum* L., *T. uncinate* Banks *et* Sol.) in order to separate the studied species microscopically, particularly among very close species within the genus.

2. Material and methods

The sample pieces have been taken from the preserved specimens from (ARB) herbarium, Biology department, College of Science, Salahaddin University - Erbil, Iraq, the sample parts were put in FAA, thereafter have been dehydrated by ascending series of alcohol concentrations (75 %, 90 %, 95 %, and 100 %), after that the samples were cleared by xylene for 3–4 h. Later, they were infiltrated in a mixture of xylene and paraffin for 30 min (paraffin and xylene 1:1 at 60C^{o}), then transferred into pure paraffin wax and left in the oven at 60^{O}C overnight. After that embedded with paraffin, blocks were made and sections were prepared with the thickness of 8 μm by rotary microtome. Then, the sections were stained using Safranin (100 ml D.W with 1 gm safranin) and fast green (100 ml %70 ethanol with 1 ml light green), Finally, the sections were mounted by DPX [23]. and examined microscopically by Olympus light microscope, the photographs were taken with a digital camera installed on the microscope.

3. Results

3.1. Transverse sections of the stem (TS)

The stem silhouette is irregular in shape as in *T. spruneriana*, *T. spicata*, and *T. monantha* var. *monantha*. It is square in *T. monspeliaca*, while semi-square in *T. caelesyriaca*. Whereas it is semi-circular such as *T. aurantiaca*, *T. strangulate*, *T. stellate* and *T. uncinate*. It is oblong in *T. filipes* and *Trigonella foenum-graecum*.

The epidermis consists of one layer, and the sclerenchyma tissue is found beneath the epidermis in all taxa. There were supportive intermittent straps of lamellar collenchyma cells in the striate areas. The collenchyma tissue is arranged circumferentially in different cell thickness layers as 4–5 layers in *T. spruneriana* and *T. stellate*, 2–3 layers in *T. spicata*, 2–5 layers in *T. monantha* var. *monantha*, 3–7 layers in *T. monspeliaca*, *T. aurantiaca* and *T. filipes*, 6–10 layers in *T. caelesyriaca*, 4–7 layers in *T. strangulate*, 5–8 layers in *T. foenum-graecum*, and 3–5 layers in *T. uncinate*. The pericyclic sclerenchyma are consolidated with the accompanying sclerenchyma as the cap of the vascular bundles consists of one ring of different layer thicknesses as 4–10 layers in *T. spruneriana*, 2–7 layers in *T. spicata*, 5–8 layers in *T. monantha* var. *monantha*, 2–9 layers in *T. monspeliaca*, 4–7 layers in *T. caelesyriaca*, 2–3 layers in *T. aurantiaca*, 6–7 layers in *T. filipes* and *T. strangulate*, 4–6 layers in *T. stellate*, 2–4 layers in *T. foenum-graecum*, 3–4 layers in *T. uncinate*. The cortex is devoid of parenchyma tissue. The stele consists of a continuous ring of compacted vascular bundles in most taxa, except in *T. caelesyriaca* which loosely arranged, while it formed incoherently from three

large vascular bundles in *T. strangulate*. Ca-oxalate crystals and tannin are found in all species. Glandular hairs were found in *T. stellate*, *T. foenum-graecum* and *T. uncinate*, non-glandular hairs are observed in *T. spruneriana*, *T. spicata* and *T. aurantiaca*, while glandular and non-glandular hairs were found on epiderms of *T. strangulate* (Figs. 1 and 2) (Table 1).

3.2. Transverse sections of the petiole (TS)

The petiole outline, adaxially is semi-concave, and abaxial surface is semi-circular as in *T. spruneriana*, *T. monantha* var. *monantha*, and *T. monspeliaca*. In *T. spicata* the adaxial is concave with abaxial is semi-rounded. The adaxial surface is semi-straight, and abaxial is "V" shaped as in *T. caelesyriaca*. In *T.*

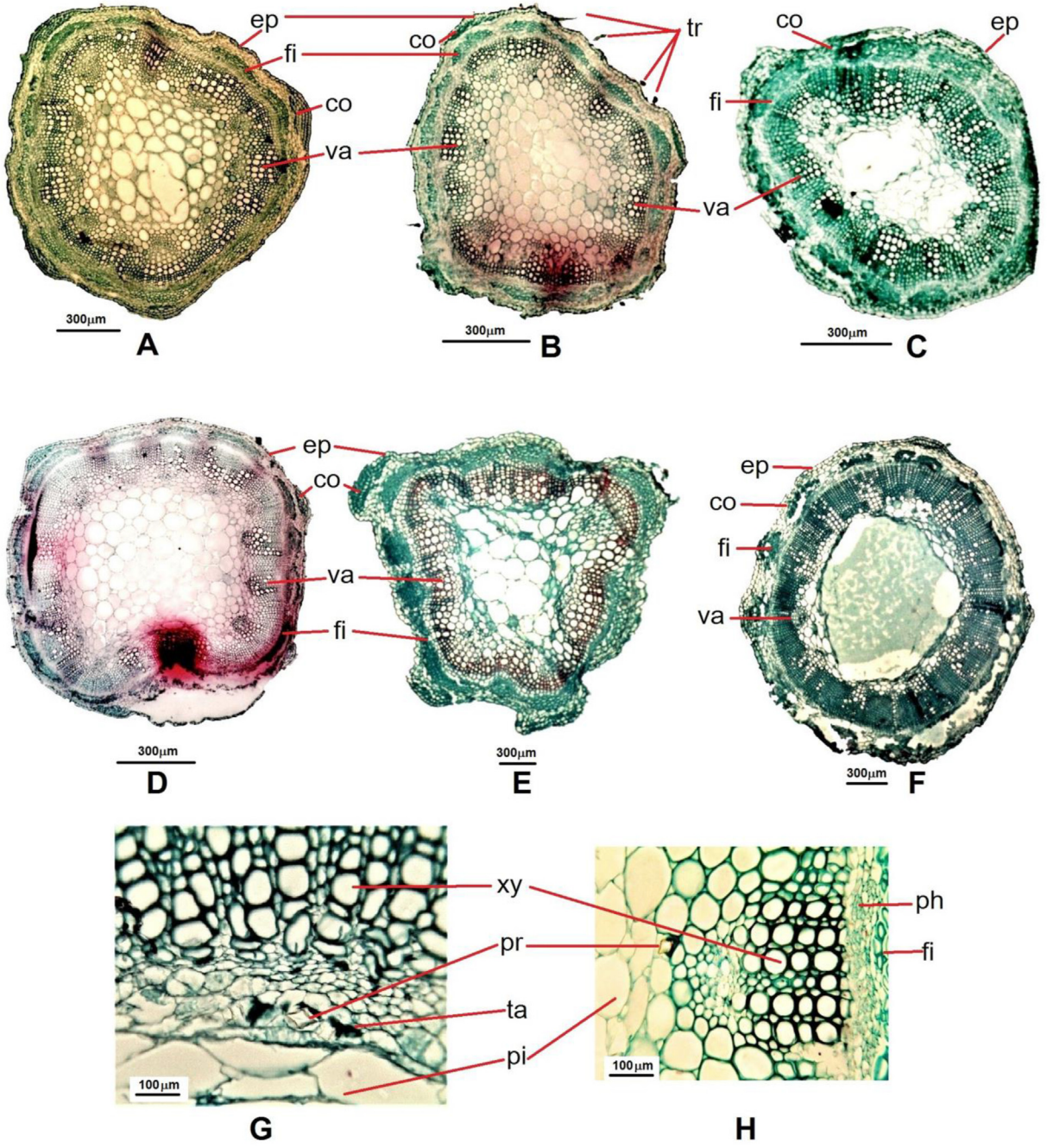

Fig. 1. TS stems. A. T. spruneriana*, B.* T. spicata*, C.* T. monantha *var.* monantha*, D.* T. monspeliaca*, E.* T. caelesyriaca*, F.* T. aurantiaca*, G-H.* T. spicata *magnified sections of stem. ep: epidermis, co: collenchyma, fi: fiber, va: vascular bundles, tr: trichomes, xy: xylem, ta: tannin, pr: prismatic crystals, pi: pith, ph: phloem. A - F = 4X (scale bars: 300 µm); G-H = 40X (scale bars: 100 µm).*

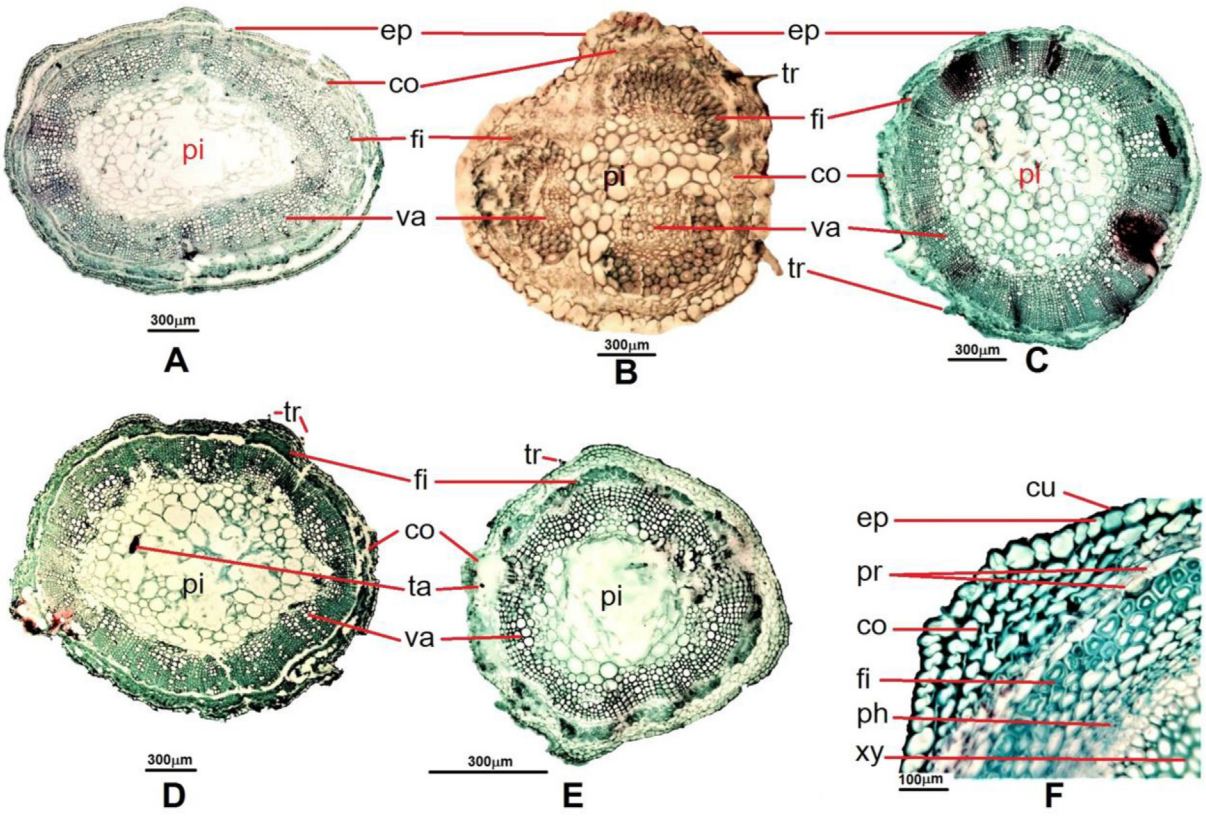

Fig. 2. TS stems. A. T. filipes*, B.* T. strangulate*, C.* T. stellate*, D.* T. foenum-graecum*, E.* T. uncinate*, F.* T. spicata *magnified section of stem. ep: epidermis, co: collenchyma, fi: fiber, va: vascular bundles, tr: trichomes, cu: cuticle, xy: xylem, ta: tannin, pr: prismatic crystals, pi: pith, ph: phloem. A - E = 4X (scale bars: 300 µm); F = 40X (scale bar: 100 µm).*

aurantiaca the adaxial surface is semi-concave, and abaxial is "U" shaped. The adaxial layer is deep concave, with abaxial layer is irregular such as *T. filipes* and *T. strangulate*, while in *T. stellate* and *T. foenum-graecum* the adaxial layer is deep concave, and abaxial layer is circular to rounded. In *T. uncinate* adaxial surface is concave with the abaxial surface is cup shaped. Non-glandular trichomes were seen in *T. spruneriana*, *T. spicata*, *T. aurantiaca*, *T. strangulate*, and *T. uncinate*, whereas the glandular trichomes were seen in *T. spruneriana* and *T. monspeliaca*. Solitary prismatic Ca-oxalate crystals and tannin are found in all species. Accessory vascular bundles found in *T. spruneriana*, *T. spicata*, *T. monantha* var. *monantha*, *T. monspeliaca*, *T. caelesyriaca*, *T. filipes*, *T. strangulate*, *T. stellate*, *T. foenum-graecum*, and *T. uncinate*, while absent in *T. aurantiaca* (Fig. 3) (Table 2).

3.3. Transverse sections of the midrib (TS)

The adaxial surface is slightly concave and abaxial is rounded to cup shaped as in *T. spruneriana*, *T. spicata*, and *T. monantha* var. *monantha*. In *T. monspeliaca* the adaxial layer is straight, abaxial layer is rounded. While in *T. caelesyriaca* the adaxial is straight with abaxial is "V" shaped. The adaxial superficies is semi-straight, and abaxial superficies is broad rounded such as *T. aurantiaca*, *T. stellate*, and *T. uncinate*. The adaxial surface is concave with abaxial is rounded as in *T. filipes*. Finally, the midrib outline is straight adaxially and rounded to broad rounded abaxially in *T. strangulate*, and *T. foenum-graecum*. The existence of the epidermal projection such as hairs are unicellular non-glandular are shown in *T. spruneriana* and *T. stellate* or unicellular glandular in *T. monantha* var. *monantha*, the presence of papillae in *T. strangulate* and *T. foenum-graecum*. Stomata apparatus were observed on the both abaxial and adaxial epidermis in all studied taxa. The solitary prismatic crystals are present in all species (Fig. 5F). The accessory vascular bundles appeared in *T. monantha* var. *monantha* and *T. aurantiaca*. The solitary prismatic crystals and tannin are present in all species (Figs. 4 and 5) (Table 2).

Table 1. Anatomical comparison of stem traits in the studied taxa.

Data \ Taxon	Stem outline shape	Number of collenchyma layers	Number of sclerenchyma layers	Vascular bundles arrangement	Glandular trichomes	Non-glandular trichomes
T. sprunerian	irregular in shape	4-5 layers	4-10 layers	continuous ring of compacted V. B	absent	present
T. spicata	irregular in shape	2-3 layers	2-7 layers	continuous ring of compacted V. B	absent	present
T. monantha var. monantha	irregular in shape	2-5 layers	5-8 layers	continuous ring of compacted V. B	absent	absent
T. monspeliaca	square	3-7 layers	2-9 layers	continuous ring of compacted V. B	absent	absent
T. caelesyriaca	semi-square	6-10 layers	4-7 layers	loosely arranged	absent	absent
T. aurantiaca	semi-circular	3-7 layers	2-3 layers	continuous ring of compacted V. B	absent	present
T. filipes	oblong	3-7 layers	6-7 layers	continuous ring of compacted V. B	absent	absent
T. strangulate	semi-circular	4-7 layers	6-7 layers	incoherently of three large V. B	present	present
T. stellate	semi-circular	4-5 layers	4-6 layers	continuous ring of compacted V. B	present	absent
T. foenum-graecum	oblong	5-8 layers	2-4 layers	continuous ring of compacted V. B	present	absent
T. uncinate	semi-circular	3-7 layers	3-4 layers	continuous ring of compacted V. B	present	absent

3.4. Transverse sections of the lamina (TS)

The leaflets lamina in all studied taxa are consists of an epidermis of a single layer, covered with a thick layer of cuticle wax. Mesophyll of two parenchymatous layers (heterogenous) of palisade and spongy parenchyma. Vascular bundles surrounded with parenchyma bundle sheath contain solitary prismatic crystals. Non-glandular trichomes were seen in *T. spruneriana*, *T. spicata*, *T. monspeliaca*, *T. strangulate*, and *T. uncinate*, while the glandular and on-glandular trichomes were seen in *T. filipes* (Fig. 6) (Table 2).

3.5. Transverse sections of the margin (TS)

The margin outline is rounded upwards such as *T. spruneriana*, *T. strangulate*, and *T. uncinate*. In *T. spicata*, *T. stellate*, and *T. foenum-graecum* is rounded straight. While pointed upwards in *T. monantha* var. *monantha*, and *T. filipes*. It is rounded downward in *T. monspeliaca*, *T. caelesyriaca*, and *T. aurantiaca*. Unicellular glandular trichomes existed in *T. monspeliaca* and *T. strangulate*, while they are unicellular non-glandular in *T. aurantiaca* and *T. foenum-graecum*. Stomata were noticed on both epidermal surfaces of the lamina in all studied taxa; however, epidermal papillae cells exist in *T. filipes*. Solitary prismatic crystals and tannin were found in all species, as formerly illustrated in the other sections (Fig. 7) (Table 2).

4. Discussion

The current investigation explores the beneficial notification of the anatomy characteristics. The critical investigation appeared the vascular bundles of stems were arranged closed in a ring, except in *T. caelesyriaca* the vascular bundles were arranged in a ring, and in *T. strangulate* were separated in patches. Siahpoosh [2]; Dural and Citak [5] pointed the vascular bundles configuration were continuous in the ring.

The critical practice shows the hairs of stems, petioles, midribs and margins were unicellular glandular or non-glandular or as papilla, and present the prosenchymatous (collenchyma tissue) at the raised protuberances beneath the single layer of the epidermis, this is consistent with [12,24]. Pereira et al., [25]; Leme and Scremin-Dias [26], reported the trichomes form were vary a lot from unicellular to multicellular may be branched or non-branched, spiral or hooked. These features

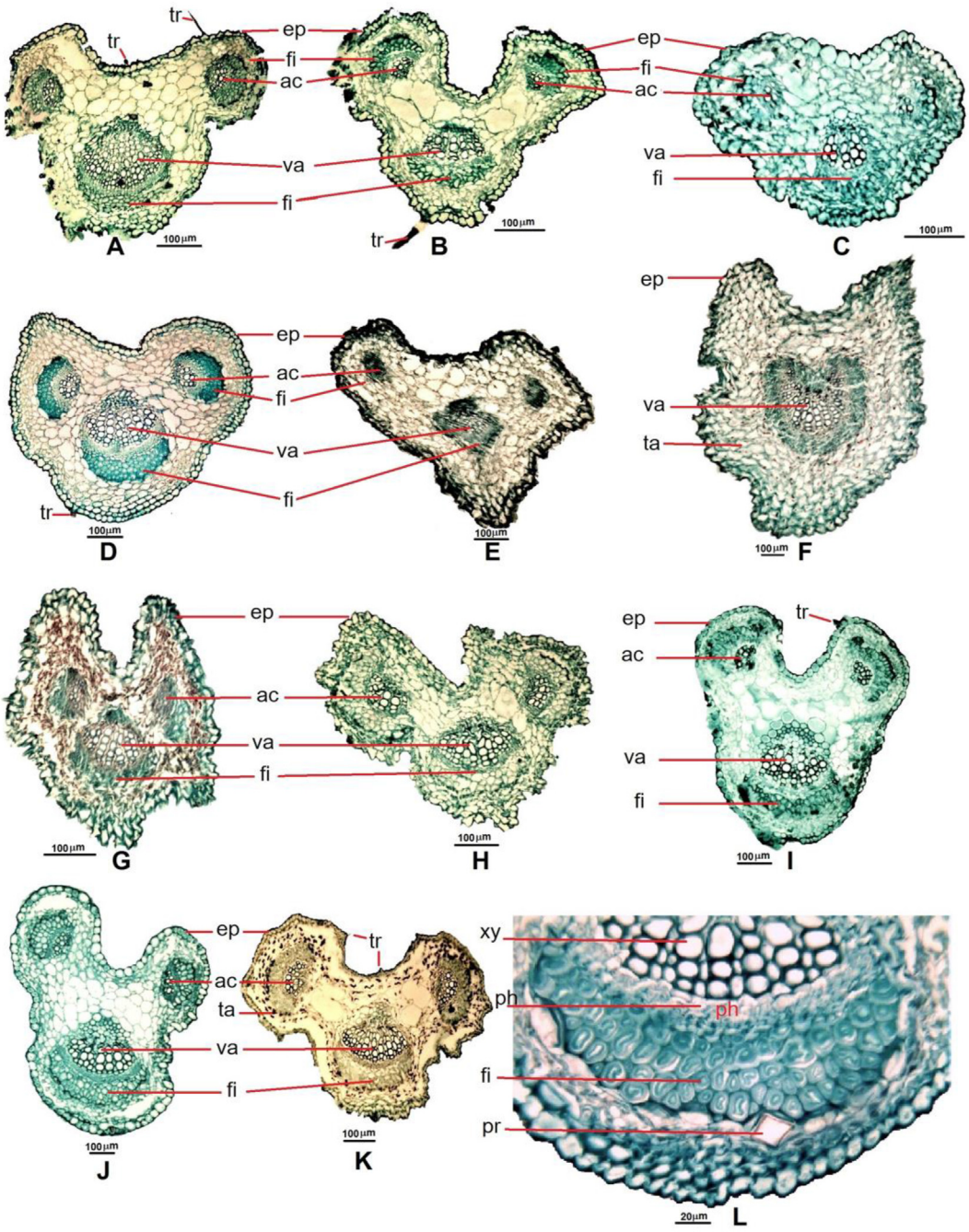

Fig. 3. TS of petioles. A. T. spruneriana, *B.* T. spicata, *C.* T. monantha *var.* monantha, *D.* T. monspeliaca, *E.* T. caelesyriaca, *F.* T. aurantiaca, *G.* T. filipes, *H.* T. strangulate, *I.* T. stellate, *J.* T. foenum-graecum, *K.* T. uncinate, *L.* T. monspeliaca *magnified section of petiole. ep: epidermis, fi: fiber, va: vascular bundles, ac: accessory vascular bundle, tr: trichomes, ta: tannin, pr: prismatic crystals, xy: xylem, ph: phloem. A - K = 4X (scale bars: 100 µm), L = 40X (scale bar: 20 µm).*

may constitute a large rating application in plant taxonomy [27].

The petiole outlines showed considerable differences among most of the studied taxa, as demonstrated by Al-dabbagh [14] the anatomical features of petioles are of taxonomic importance and can contribute to delimiting the species of certain genera within a Fabaceae family. The critical

Table 2. Anatomical comparison of leaf traits in the studied taxa.

Data \ Taxon	Petiole		Midrib		Margin outline	Glandular trichomes	Non-glandular trichomes
	Petiole outline shape	Accessory V. B	Midrib outline shape	Accessory V. B			
T. sprunerian	Adaxially is semi-concave, and abaxial surface is semi-circular	present	Adaxial is slightly concave and abaxial is rounded to cup shaped	absent	Rounded upwards	Petiole	Petiole and lamina
T. spicata	Adaxial is concave with abaxial is semi-rounded	present	Adaxial is slightly concave and abaxial is rounded to cup shaped	absent	Rounded straight	- - - -	Petiole, midrib and lamina
T. monantha var. monantha	Adaxially is semi-concave, and abaxial surface is semi-circular	present	Adaxial is slightly concave and abaxial is rounded to cup shaped	present	Pointed upwards	Midrib	- - - -
T. monspeliaca	Adaxially is semi-concave, and abaxial surface is semi-circular	present	Adaxial is straight, abaxial layer is rounded	absent	Rounded downward	Petiole and margin	Lamina
T. caelesyriaca	Adaxial surface is semi-straight, and abaxial is “V” shaped	present	Adaxial is straight with abaxial is “V” shaped	absent	Rounded downward	- - - -	Petiole
T. aurantiaca	Adaxial surface is semi-concave, and abaxial is “U” shaped	absent	Adaxial is semi-straight, and abaxial is broad rounded	present	Rounded downward	- - - -	Margin
T. filipes	Adaxial layer is deep concave, with abaxial layer is irregular	present	Adaxial is concave with abaxial is rounded	absent	Pointed upwards	Lamina	Lamina
T. strangulate	Adaxial layer is deep concave, with abaxial layer is irregular	present	Straight adaxially and rounded to broad rounded abaxially	absent	Rounded upwards	Margin	Petiole and midrib
T. stellate	Adaxial layer is deep concave, and abaxial layer is circular to rounded	present	Adaxial is semi-straight, and abaxial is broad rounded	absent	Rounded straight	- - - -	Midrib
T. foenum-graecum	Adaxial layer is deep concave, and abaxial layer is circular to rounded	present	Straight adaxially and rounded to broad rounded abaxially	absent	Rounded straight	- - - -	Margin
T. uncinate	Adaxial surface is concave with the abaxial surface is cup shaped	present	Adaxial is semi-straight, and abaxial is broad rounded	absent	Rounded upwards	- - - -	Petiole and margin

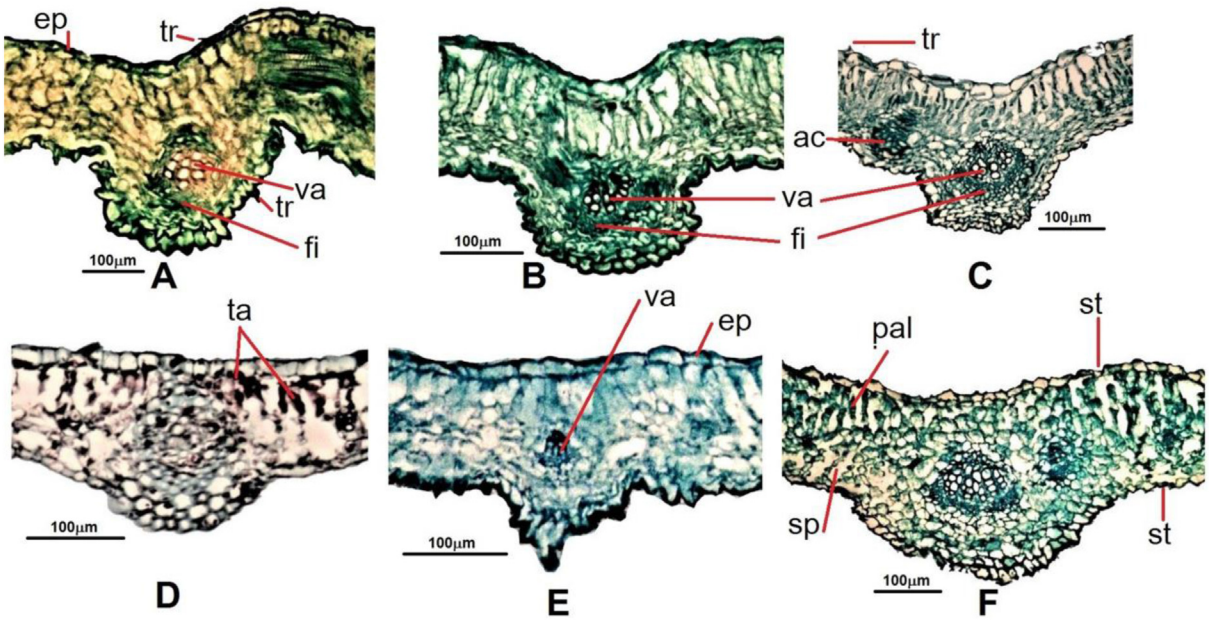

Fig. 4. TS of midribs. A. T. spruneriana*, B.* T. spicata*, C.* T. monantha *var.* monantha*, D.* T. monspeliaca*, E.* T. caelesyriaca*, F.* T. aurantiaca*. ep: epidermis, fi: fiber, va: vascular bundles, ac: accessory vascular bundle, st: stoma, tr: trichomes, ta: tannin, pa: papillae, pal: palisade parenchyma, sp: spongy parenchyma, pr: prismatic crystals, xy: xylem, ph: phloem. A - F = 4X (scale bars: 100 µm).*

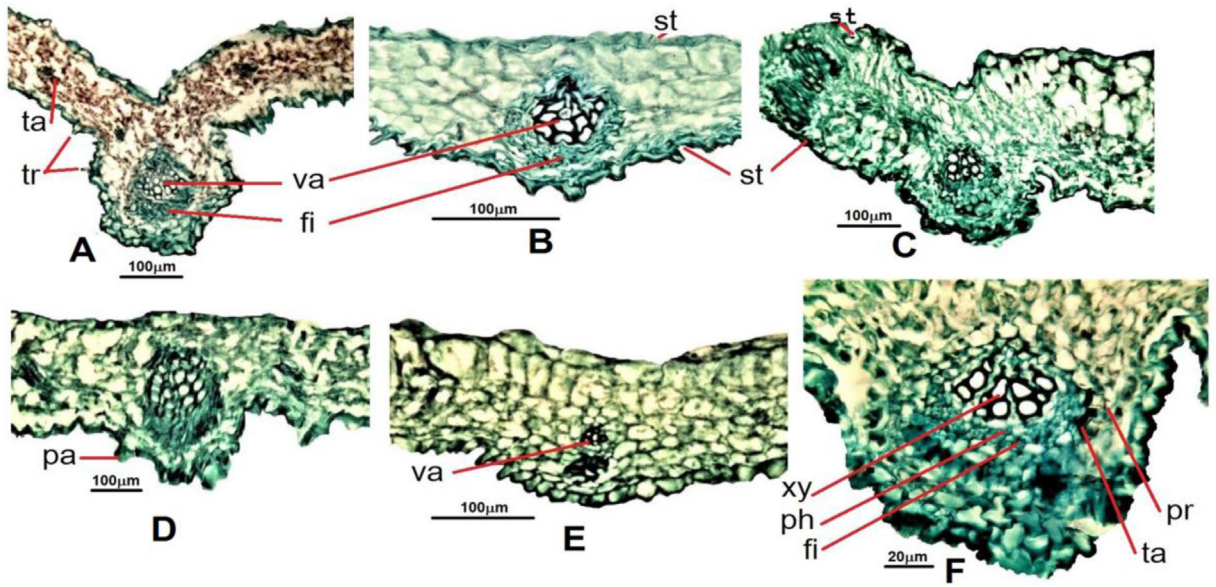

Fig. 5. TS of midribs. A. T. filipes*, B.* T. strangulate*, C.* T. stellate*, D.* T. foenum-graecum*, E.* T. uncinate*, F.* T. monantha *var.* monantha *magnified section of midrib. ep: epidermis, fi: fiber, va: vascular bundles, ac: accessory vascular bundle, st: stoma, tr: trichomes, ta: tannin, pa: papillae, pal: palisade parenchyma, sp: spongy parenchyma, pr: prismatic crystals, xy: xylem, ph: phloem. A - E = 4X (scale bars: 100 µm), F = 40X (scale bar: 20 µm).*

analysis displayed the presence of accessory vascular bundles except for *T. aurantiaca* and fibers in the petioles of all species, this argument agrees with the presence of accessory bundles in petioles [2]. The lamina of the leaflets showed a great similarity among the species in the characteristics of their mesophyll content, and therefore the blade is considered invaluable in the separation of the

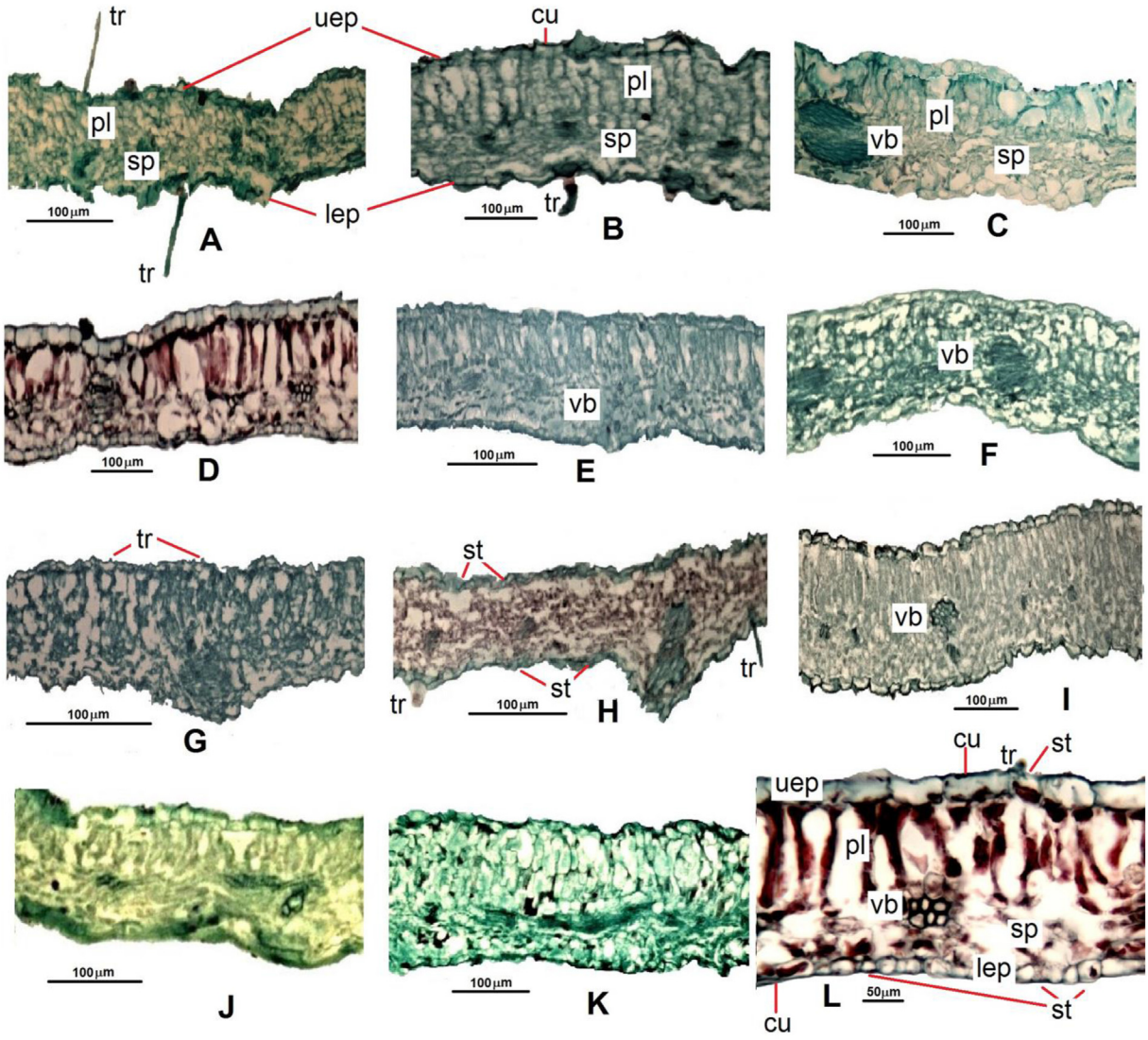

Fig. 6. TS of leaflets lamina. A. T. spruneriana*, B.* T. spicata*, C.* T. monantha *var.* monantha*, D.* T. monspeliaca*, E.* T. caelesyriaca*, F.* T. aurantiaca*, G.* T. filipes*, H.* T. strangulate*, I.* T. stellate*, J.* T. foenum-graecum*, K.* T. uncinate*, L.* T. monspeliaca *magnified section of leaflet margin. cu: cuticle, uep: upper epidermis, lep: lower epidermis, pl: palisade mesophyll, sp: spongy mesophyll, st: stoma, tr: trichomes, pr: prismatic crystals, bs: bundle sheath, and vb: vascular bundles. A - K = 10X (scale bars: 100 µm), L = 40X (scale bar: 50 µm).*

studied species. Although, there are slight differences in the lamina epidermis containment of glandular and non-glandular hairs. The solitary prismatic crystals are found in all parts of the plants as referred by [28]. The ergastic substances as tannins found in all parts of the samples; this analysis agreement with [29].

5. Conclusion

It was concluded in the current research the following, the anatomical features of the stems showed clear variations in terms of the outlines of the stems, and some species were unique to these characters in view of, being manifested in different forms. The outlines of the leaf petiole, midrib and margins have explicit variations that could be relied upon to separate the studied species of the genus. Depending on more than one anatomical trait, it is possible to construct an anatomical taxonomic key for delimiting among very similar phenotypic species. In addition to all these variations, there are common features that were noted, such as the characteristics of the appearance of tannin and

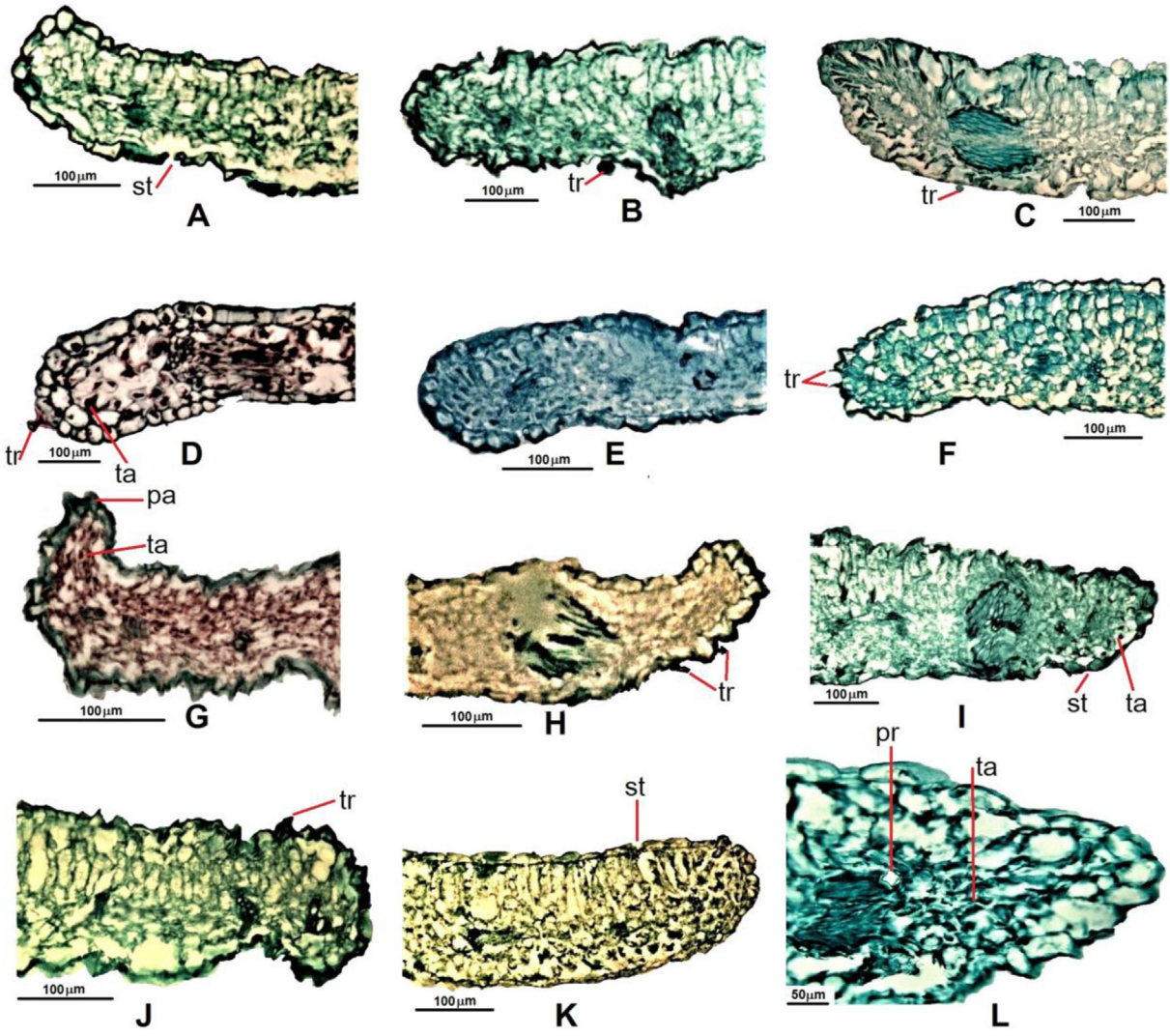

Fig. 7. TS of margins. A. T. spruneriana*, B.* T. spicata*, C.* T. monantha *var.* monantha*, D.* T. monspeliaca*, E.* T. caelesyriaca*, F.* T. aurantiaca*, G.* T. filipes*, H.* T. strangulate*, I.* T. stellate*, J.* T. foenum-graecum*, K.* T. uncinate*, L.* T. stellate *magnified section of leaf margin. st: stoma, tr: trichomes, ta: tannin, pa: papillae, pr: prismatic crystals. A - K = 4X (scale bars: 100 µm), L = 40X (scale bar: 50 µm).*

solitary prismatic crystals in the different parts of all the studied species, it appears that these characteristics specific to the genus or even a Fabaceae family.

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Conflict of interest

There is no conflict of interest.

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