ANATOMICAL VARIATIONS OF LEAVES PETIOLES IN SOME TAXA OF THE GENUS *TRIFOLIUM* L. (FABACEAE) IN IRAQ.

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ABSTRACT

This study was conducted on the 22 taxa of the genus *Trifolium* L. which grow naturally as wild plants in all geographical districts in Iraq. A comparative study of the leaf petioles was anatomically examined by paraffin method. Petiole cross-section outline shapes have divided the genus species into fourteen groups and sub-groups. According to the microscopical characteristics the cortex of petioles showed to contain layers of various tissues, the collenchyma tissue observed sub-epidermally in some species, and cortexes of the others were devoid of collenchyma. The sclerenchyma strands were noted in three patterns; above vascular bundles, above and beneath vascular bundles, and the sclerenchyma strands completely surrounded the vascular bundles in some species. Petiole cross-sections have been divided into three categories as; three vascular bundles and absent of accessory vascular bundles, three vascular bundles with one of accessory vascular bundle, three vascular bundles with two accessory vascular bundles. The comparative results confirmed that these characteristics collectively are of high taxonomic value and can contribute to separating the studied genus species, finally, based on these variations, the anatomical classification key was constructed to separate the genus taxa.

Key word: collenchyma tissue, crystals, glandular hairs, leaves stalks, vascular bundles.

التغايرات التشريحية لسويقات الأوراق في بعض انواع الجنس *Trifolium L* . (Fabaceae) في العراق.

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مدرس

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المستخلص

أجريت هذه الدراسة على 22 نوع من جنس الـ *Trifolium* L. والتي تنمو و تنتشر بشكل بري و طبيعي في جميع المناطق الجغرافية في العراق. تمت دراسة مقارنة لسويقات الأوراق تشريحيا بطريقة البارافين. أشكال الخطوط االمحيطية للمقطع العرضي للسويقات قسمت أنواع الجنس إلى أربعة عشرة مجموعة رئيسية ومجموعات فرعية. وفقًا للخصائص المجهرية، أظهرت قشرة السويقات إحتوائها على طبقات مختلفة من الأنسجة، لوحظ وجود النسيج الكولنكيمي تحت البشرة في بعض الأنواع، في حين كانت خالية تماما في الانواع الاخرى. لوحظت طبقات الالياف السكلرنكيمية في ثلاثة أنماط؛ فوق الحزم الوعائية و فوق وتحت الحزم الوعائية وخيوط سكلرنكيمية تحيط بالحزم كاملا في بعض الأنواع. تم تقسيم المقاطع العرضية المطوية إلى ثلاث فئات على النحو التالي: ثلاث حزم وعائية فقط وغياب الحزم الوعائية المساعدة، وثلاث حزم وعائية مع حزمة وعائية مساعدة ، وثلاث حزم وعائية مع حزمتين من الحزم الوعائية المساعدة. أكدت النتائج المقارنة أن هذه الصفات مجتمعة ذات قيمة تصنيفية عالية ويمكن أن تساهم في فصل الأنواع المدروسة للجنس، و بالاعتماد على هذه المتغيرات تم إنشاء مفتاح تصنيفي تشريحي لفصل الأنواع.

الكلمات المفتاحية: نسيج كولنكيمي، بلورات، الشعيرات الغدية، اعناق الاوراق، الحزم الوعائية.

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INTRODUCTION

Fabaceae (Leguminosae) also called the pea family or beans (faba, the Latin name for broad beans), comprises about 18,000 species within 643 genera. Most taxa of the family are of great agricultural food value, plants are herbs, shrubs, trees, or vines, some taxa are spiny plants. The family members are prevailing species in certain ecosystems and have ecologically remarkable importanance due to rhizobial nodules for nitrogen-fixing, most species are cultivated in plant rotation for this purpose. The *Trifolium* spp. are considered as forage, pasture, and soil fodder in plant rotations (9, 24, 29). The family is distinguished by its floral and fruity characteristics (19). The *Trifolium* is the most widespread genus among the genera of the family (16), especially about 103 species widely distributed in Turkey (12). The cultivated forage species with high forage importance in Europe and Serbia are *T. hybridum* L., *T. repens* L., *T. incarnatum* L., *T. pratense* L., *T. subterraneum* L. and *T. resupinatum* L. (8, 9, 15). Taia (26) studied *Trigonella*, *Ononis*, *Trifoliun*, *Melilotus*, and *Medicago* seeds with a view of assessing the relationship among them. He managed to separate *Trifolium* and *Ononis* into separate sub-tribes (30), *Trifolium* species are widely cultivated as forage plants, and some species are used as antiseptics, expectorants, analgesics, and for rheumatic pain as Turkish herbal medicine (22). Many clinical and pre-clinical works investigated the multiple actions associated with *Trifolium* and its isoflavones action that reflect estrogenic, anti-estrogenic, and independent estrogenic processes (7). In Iraq, some botanists have calculated the taxa of the genus *Trifolium* as Zohary (28) mentioned 17 species; Townsend and Guest (27) cited 36 species, sub-species and varieties; Ridda and Daood (21) counted 38 species, sub-species and varieties; Al- Rawi (5) reported 33 species, sub-species and varieties; Darwesh (11) pointed out the presence of 11 species in Choman-Erbil district. All these species grow naturally as wild plants in different districts in Iraq.Plant anatomy is a major fundamental science to all botanists, the detailed study of the plant components and tissue structures enables a better comprehension of acclimatization to different environmental effects (14). The anatomical criterion may play a significant role in plant classification (17). The anatomy of vegetative parts of the *Trifolium* taxa have not been studied widely, whilst most anatomical studies were directed towards agricultural and economical important species, of which the most examined species was *T. repens* (31). Microscopic features of some *Trifolieae* species contribute to understanding their relationship and for better separation (3, 26). Anatomical characteristics can be applied to species diagnostic (23). The cross-sections of petioles play an important role in determining the species in the same genus (6). A detailed analysis of the anatomical variations of *Trifolium* taxa leaf petioles has not been previously performed, regardless of the other vegetative parts. Therefore, in the present work, a comparison of microscopic characteristics of the *Trifolium* taxa petioles has been conducted, in order to verify the anatomical characteristic that could be useful for classifying and constructing a taxonomic key for an anatomical separation of studied taxa.

MATERIALS AND METHODS

The plant material was taken either from fresh samples collected during the spring and summer of 2020 or from dry samples which were deposited in the herbarium of the College of Science, Salahaddin University - Erbil (ARB). A portion of each petiole about 0.5-1 cm^2 was cut and fixed in FAA (formalin, acetic acid and alcohol). The paraffin method was performed as stated by Al-dabbagh (1) to prepare the permanent slides for tissue samples as follows; The samples were dehydrated by using series concentrations (30%, 50%, 60%, 75%, and absolute) of ethyl alcohol, then the samples were cleared by xylene for 3-4 hrs. (3times). After that, the embedding of samples was done by immersing the plant samples in paraffin and left for a night in the oven at 60^{O}C. The blocks of paraffin were made by certain metal blocks. Slide sections have been cut with the thickness of 6-8 µm by using the rotary microtome (Bright, LTD), the ribbons were placed and mounted on clean slides carefully, then moved onto a hot plate for a night. The slides were stained by safranin

(3hrs) and fast-green (0.5-1) min., a drop of DPX was added and covered by coverslips. The slides have been inspected and pictured by Light Microscopes (Olympus AC 100 with a mounted camera, Japanese-made). The used anatomical terms were cited by (10,13, 14, 18,).

RESULTS AND DISCUSSION

Cross sections outline ″Figure 1″ and ″Table 1″: Microscopic cross-sections of petioles were different in outline shape, and that was determined by the depth of emargination on the adaxial side and the degree and shape of the abaxial curving. Petiole cross-sections in some species were heart-shaped with differences in their emargination depth such as; heart-shaped deeply invaginate on the adaxial to V-shaped in *T. ambiguun* M. Bieb. (Fig 1A); heart-shaped to pyriform in *T. campestre* Schreb. (Fig 1C); heart-shaped in *T. cherleri* L. (Fig 1D); polygonal heart-shaped in *T. dasyurum* Presl. (Fig 1E); broadly heart-shaped undulate with distinct prominence at adaxial in *T. fragiferum* L. (Fig 1G); triangular to heart-shaped in *T. hirtum* All., Auct. (Fig 1J) and *T. pilulare* Boiss (Fig 2C); heart-shaped, deeply emarginated at adaxial forming broad V-shaped in *T. lappaceum* L. (Fig 1K), and broadly heart-shaped in *T. scabrum* L. (Fig 2F). Semi-circular shallowly emarginated on adaxial forming apple-shaped (malum informibus) in *T. arvense* L. (Fig 1B). Others reniform as *T. grandiflorum* Schreb. (Fig 1H), *T. guestii* Blackelock (Fig 1I), and *T. tomentosum* var *glabrescens* (Post) Hausskn (Fig 2J), while narrowly reniform to crescent-shaped in *T. echinatum* M. Bieb. (Fig 1F). Narrowly lunate in *T. leucanthum* M. Bieb. (Fig 1L). Some petiole cross-sections were in different forms of V-shaped as; broadly V-shaped in *T. nigrescens* subsp. *nigrescens* Viv., Fragm. (Fig 2A), and *T. resupinatum* L. (Fig 2E); broadly V-shaped, the vascular bundle sites are clearly visible in *T. physodes* var *glabrescens* Stev. Ex M. Bieb. (Fig 2B), whereas V-shaped in *T. spumosum* L. (Fig 2G), and *T. stellatum* var *stellatum* L. (Fig 2H). The remaining two petiole cross-sections were; Y-shaped (elongated abaxial with two divaricate long projections) in *T. purpureum* Lois. (Fig 2D), and pear-shaped (pyriform) oblong to panduriform in *T. sylvaticum* Gér. ex Lois. (Fig 2I). The cross-sections of the examined petioles revealed that the cross-sections of petioles are the best characters for delimiting among certain genus taxa (1, 19). Additionally, through it, these shapes can be divided into six groups (heart-shaped, semi-circular, reniform, Pear-shaped, V-shaped, and lunate), and these groups can be divided into sub-groups and so on. There were four specific petiole cross-section outlines observed, which were exclusive in some taxon, they were; apple-shaped, narrowly lunate, Y-shaped, and pear-shaped.

General descriptions of petioles ″Figure3″

The epidermis in all studied taxa was one layer comprised of small thick-walled epidermal cells, approximately similar in shape and size, the stomata are usually present. Unicellular capitate glandular trichomes were observed in *T. fragiferum*, *T. scabrum*, and *T. spumosum* (Fig 3A). Unicellular unbranched non-glandular trichomes were observed in most species such as *T. arvense*, *T. campestre*, *T. cheleri*, *T. dasyurum*, *T. echinatum*, *T. hirtum*, *T. stellatum* var *stellatum*, and *T. sylvaticum* (Fig 3B). Both unicellular capitate glandular and simple unicellular unbranched non-glandular trichomes were present on epidermis in some species as *T. guestii*, *T. leucanthum*, and *T. purpureum* (Fig 3C). The rest of the species were glabrous (Fig 3D), while the tannin substance occupied most of the epidermal cell lumens in *T. arvense* (Fig 3B). Hypodermis comprises 1 layer of lacunar collenchyma sub-epidermally in *T. dasyurum*, *T. fragiferum*, *T. guestii*, *T. resupinatum*, and *T. tomentosum* var *glabrescens*, 1 layer of lamellate collenchyma in *T. hirtum*, 1-2 cells thickened lacunar collenchyma tissue in *T. echinatum*, and *T. sylvaticum*, 2-3 cell thickened of lacunar collenchyma tissue in *T. arvense*. The cortex is supplemented with 2-4 layers of parenchymatous tissue containing chloroplasts inward to the vascular bundle sheaths, then it continues as a mass of parenchymatous tissue inward to the central pith. Whereas the rest of the species were devoid of collenchyma tissue. The cortexes of these species comprise only 2-5 layers of parenchymatous tissue to the vascular bundles sheath instead, and completed with a mass of

parenchymatous tissue inward to the pith (Fig 3A-F). The epidermal cells showed various anatomical features, simple glandular and non-glandular trichomes were seen together in some species, some others exhibited only one type, and the trichomes were absent in epidermis of the rest species. The trichomes are a taxonomic value at the species level, even to varieties level in plant delimitation, this agrees with (1, 2, 20, 25). While, *T. arvense* is the only species that contains tannin in epidermal cells, this attribute was not mentioned in previous researches. Subdermally, the hypodermis was supported by 1-3 layers of collenchyma in some species, in contrast, the cortex was devoid of collenchyma in others (14, 19).

Vascular bundles pattern ″Figure 1″

All vascular bundles have ovate or broadly ovate shapes in cross-section, surrounded with bundle sheath of a layer of parenchyma cells. Usually, solitary prismatic crystals are occurring in bundle sheath cells adjacent to the sclerenchyma strains. Three types of bundles were noted in examined petiole cross-sections according to the sclerenchyma location; the first type was 2-6 layers of sclerenchyma cells crescent-like or cap-shaped above vascular bundles as *T. ambiguun*, *T. campestre*, *T. grandiflorum*, *T. lappaceum*, *T. nigrescens* subsp. *nigrescens*, *T. physodes* var *glabrescens*, *T. pilulare*, *T. resupinatum*, *T. spumosum*, and *T. tomentosum* var *glabrescens* (Fig 3D); the second type was 5-6 layers of sclerenchyma cells crescent-like above the vascular bundles, with 2-5 layers cup-shaped under the vascular bundles such as *T. arvense*, *T. cherleri*, *dasyurum*, *T. echintum*, *T. echinatum*, *T. hirtum*, *T. purpureum*, and *T. sylvaticum* (Fig 3B); the third type, 3-6 sclerenchyma strands were well developed and completely surrounded the vascular bundles, usually, the layers under bundles and at both sides were less, observed in *T. guestii*, *T. leucanthum*, *T. scabrum*, and *T. stellatum* var *stellatum*. The arrangement, distribution, and numbers of vascular bundles in the cross-section of petioles differed between the studied species. In some species three vascular bundles were observed as one large with two smaller and similar in *T. campestre* (Fig 1C); one large at abaxial with two small vascular bundles at divaricated sites in *T. purpureum* (Fig 2D); one large at abaxial with two smaller similar in *T. sylvaticum* (fig 2I); while three vascular bundles are approximately similar in size and shape were noted in *T. grandiflorum*(Fig 1H), and *T. guestii* (Fig 1I). Moreover, four vascular bundles, three large and approximately similar, with one accessory in one side were recognized in *T. cherleri* (Fig 1D), *T. pilulare* (Fig 2C), and *T. tomentosum* var *glabrescens* (Fig 2J). Whereas most of the studied species were distinguished with five variable vascular bundles as three large with two similar accessories in *T. ambiguun* (Fig 1A); three large similar, with two similar accessories in *T. dasyurum* (Fig 1E), and *T. echinatum* (Fig 1F); one large at abaxial, with four semi-similar bundles in *T. fragiferum* (Fig 1G); three large similar with two similar accessories in *T. hirtum* (Fig 1J), *T. lappaceum* (Fig 1K), *T. nigrescens* subsp. *nigrescens* (Fig 2A), *T. resupinatum* (Fig 2E), *T. stellatum* var *stellatum* (Fig 2H), and *T. scabrum* (Fig 2F); three large bundles similar with two accessories very close to the abaxial one in *T. leucanthum* (Fig 1L); three large bundles, the largest one at abaxial, two medium at terminal sites of divarication projections with two accessory alternating large vascular bundles in *T. physodes* var *glabrescens* (Fig 2B); three large bundles with two accessories almost larger than accessory vascular bundles of the other species in *T. spumosum* (Fig 2G). A single species *T. arvense* was distinguished with a discontinuous ring of seven various size vascular bundles (Fig 1B). The arrangement of the vascular bundles has differed in the petiole of different species. They appear as an interrupted ring or crescent shaped (14) containing 3-7 vascular bundles. Considering the variability of the vascular bundles and the vascular pattern in the leaf petiole, most species were separated (31), the petioles of compound leaf in the genus *Trifolium* has played a great role in classification (17). The petiole vascular bundle arrangement pattern and presence or absence of trichomes are of high taxonomic value (1, 2). The petiole supporting tissues are sclerenchyma and/or collenchyma (14), the vascular bundles are accompanied by sclerenchyma strands in all examined species, with some differences in

position (3). There are some species that contain fibers above the vascular bundles, and some of them show the presence of fibers on the upper and lower sides of the vascular bundles, while in others the fibers completely surrounded the vascular bundles. Crystals were few and only solitary prismatic type noted in all *Trifolium* species, taxonomically, the type and distribution of the crystals in the studied species were not of valuable characteristic. It might be more helpful at the level of genus or even family and this is consistent with what cited by other researchers (4, 32). Based on the characteristics obtained in the current study, an anatomical classification key was constructed for the studied species of the genus *Trifolium*, as below:

Anatomical key to species

1. Cross-section outline heart-shaped **2**
1. Cross-section outline not heart-shaped..**11**
2. Vascular bundles 5 . . . . . .**3**
2. Vascular bundles less, 3 or 4 . . . . . **8**
3. trichomes absent **4**
3. trichomes present **5**
4. Heart-shaped oblong, invaginated to V-shaped, trichomes absent . . . . . ***T. ambiguun***
4. Broadly heart-shaped, invaginated to V-shaped, trichomes absent . . . . . ***T. lappaceum***
5. Trichomes are glandular **6**
5. Trichomes are non-glandular **7**
6. Broadly heart-shaped, with distinct prominence at adaxial, glandular trichomes present . . . . . ***T. fragiferum***
´6. Broadly heart-shaped to triangular, glandular trichomes present . . . . ***T. scabrum***
7. Polygonal heart-shaped, non-glandular trichomes present . . . . . ***T. dasyurum***
7. Triangular to heart-shaped, non-glandular trichomes present . . . . . ***T. hirtum***
8. Vascular bundles 4 . . . . . **9**
8. Vascular bundles 3 . . . . . **10**
9. Triangular to heart-shaped, sclerenchyma strands above and beneath vascular bundles, non-glandular trichomes present . . . . . ***T. cherleri***
9. Triangular to heart-shaped, sclerenchyma strands only above vascular bundles, trichomes absent . . . . . ***T. pilulare***
10. Heart-shaped to pyriform, non-glandular trichomes present . . . . . ***T. campestre***
11. Cross-section outlines variously shaped, not heart-shaped **12**
11. Semi-circular shallowly emarginated at adaxial to apple-shaped, vascular bundles 7, epidermis cells occupied with tannin, non-glandular trichomes present . . . . . ***T. arvense***
12. Cross-section outlines reniform **13**
12. Cross-section outlines (V- or Y-) shaped **17**
13. Narrowly reniform to crescent shape, vascular bundles 5, non-glandular trichomes present, lacunar collenchyma sb-epidermally, above and beneath vascular bundles . . . . . ***T. echinatum***
13. Reniform, vascular bundles 3 or 4 . . . .**14**
14. Reniform, vascular bundles 3 . . . . . **15**
14. Reniform, vascular bundles 4 . . . . . **16**
15. Reniform, vascular bundles 3, trichomes absent, devoid of collenchyma, sclerenchyma strands above vascular bundles . . . . . ***T. grandiflorum***
15. Reniform, vascular bundles 3, glandular and non-glandular trichomes present, lacunar collenchyma sb-epidermally, sclerenchyma strands are completely surround the vascular bundles . . . . . ***T. guetsii***
16. Reniform, vascular bundles 4, trichomes absent, lacunar collenchyma sb-epidermally, sclerenchyma strands above vascular bundles . . . . . ***T. tomentosum* var *glabrescens***
16. Pyriform oblong to panduriform, vascular bundles 3, non-glandular trichomes present, lacunar collenchyma sb-epidermally, above and beneath vascular bundles . . . . . ***T. sylvaticum***
17. Cross-section outlines V-shaped **18**
17.Cross-section outlines Y-shaped **22**
18. V-shaped **19**
18. Broadly V-shaped **20**
19. V-shaped, vascular bundles 5 (3 similar larges with 2 similar accessories almost larger than other accessories), glandular trichomes are present, 4- 5 layers of sclerenchyma above vascular bundles . . . . . ***T. spumosum***
19. V-shaped, vascular bundles 5 (3 similar larges with 2 similar accessories), non-glandular trichomes are present, sclerenchyma strands completely surround vascular bundles . .***T. stellatum* var *stellatum***
20. Broadly V-shaped, vascular bundles 5, similar in size and shape **21**

20. Broadly V-shaped the sites of the vascular bundles are visible, vascular bundles 5 (3 larges, the largest one at abaxial and two mediums at the terminal of divaricate with 2 similar accessories), trichomes absent, sclerenchyma above vascular bundles . . . . . ***T. physodes* var *glabrescens***

21. Broadly V-shaped, trichomes absent, 5- 6 layers of sclerenchyma above vascular bundles . . . ***T. nigrescens* subsp. *nigrescens***

21. Broadly V-shaped, trichomes absent, 3- 4 layers of sclerenchyma above vascular bundles . . . . . ***T. resupinatum***

22. Y-shaped, elongated abaxial with divaricate long projections, vascular bundles 3, glandular and non-glandular trichomes are present . . . . . ***T. purpureum***

22. Narrowly lunate, vascular bundles 5, glandular and non-glandular trichomes present, sclerenchyma strands are completely surround the vascular bundles . . . . . ***T. leucanthum***

Table 1. outline and internal features of studied species petiole cross-sections

Data / Species	Cross-section outline	V. B No.*	Trichomes	Sclerenchyma layers
T. ambiguun	Heart-shaped to V-shaped	5	Absent	5 layers above V. Bs*
T. arvense	Semi-circular to apple-shaped	7	Non-glandular	5-6 layers above with 2 layers under V. Bs
T. campestre	Heart-shaped to pyriform	3	Non-glandular	2-3 layers above V. Bs
T. cherleri	Heart-shaped	4	Non-glandular	4-5 layers above with 2-3 layers under V. Bs
T. dasyurum	Polygonal heart-shaped	5	Non-glandular	3-6 layers above with 2-3 layers under V. Bs
T. echinatum	Narrowly reniform to crescent-shaped	5	Non-glandular	4-5 layers above with 2-3 layers under V. Bs
T. fragiferum	Broadly heart-shaped undulate with distinct prominence at adaxial	5	Glandular	6-7 layers above with 2-3 layers under V. Bs
T. grandiflorum	Reniform	3	Absent	4-5 layers above V. Bs
T. guestii	Reniform	3	Glandular and Non-glandular	4-5 layers completely surrounds V. Bs
T. hirtum	Triangular to heart-shaped	5	Non-glandular	6-7 layers above with 2-3 layers under V. Bs
T. lappaceum	Heart-shaped to broad V-shaped	5	Absent	4-5 layers above V. Bs
T. leucanthum	Narrowly lunate	5	Glandular and Non-glandular	6-7 layers completely surrounds V. Bs
T. nigrescens* subsp. *Nigrescens	Broadly V-shaped	5	Absent	5-6 layers above V. Bs
T. physodes* var *glabrescens	Broadly V-shaped, the V. B sites are clearly visible	5	Absent	5-7 layers above V. Bs
T. pilulare	Triangular to heart-shaped	4	Absent	3-4 layers above V. Bs
T. purpureum	Y-shaped	3	Glandular and Non-glandular	3-5 layers above with 2-4 layers under V. Bs
T. resupinatum	Broadly V-shaped	5	Glabrous	4-5 layers above V. Bs
T. scabrum	Broadly heart-shaped	5	Glandular	completely surrounds V. Bs
T. spumosum	V-shaped	5	Glandular	3-4 layers above V. Bs
T. stellatum* var *stellatum	V-shaped	5	Non-glandular	5-6 layers above with 3-4 layers under V. Bs
T. sylvaticum	Pear-shaped oblong to panduriform	3	Non-glandular	4 layers above with 3-4 layers under V. Bs
T. tomentosum* var *glabrescens	Reniform	4	Absent	4-5 layers above V. Bs

*** V. B = Vascular bundles**

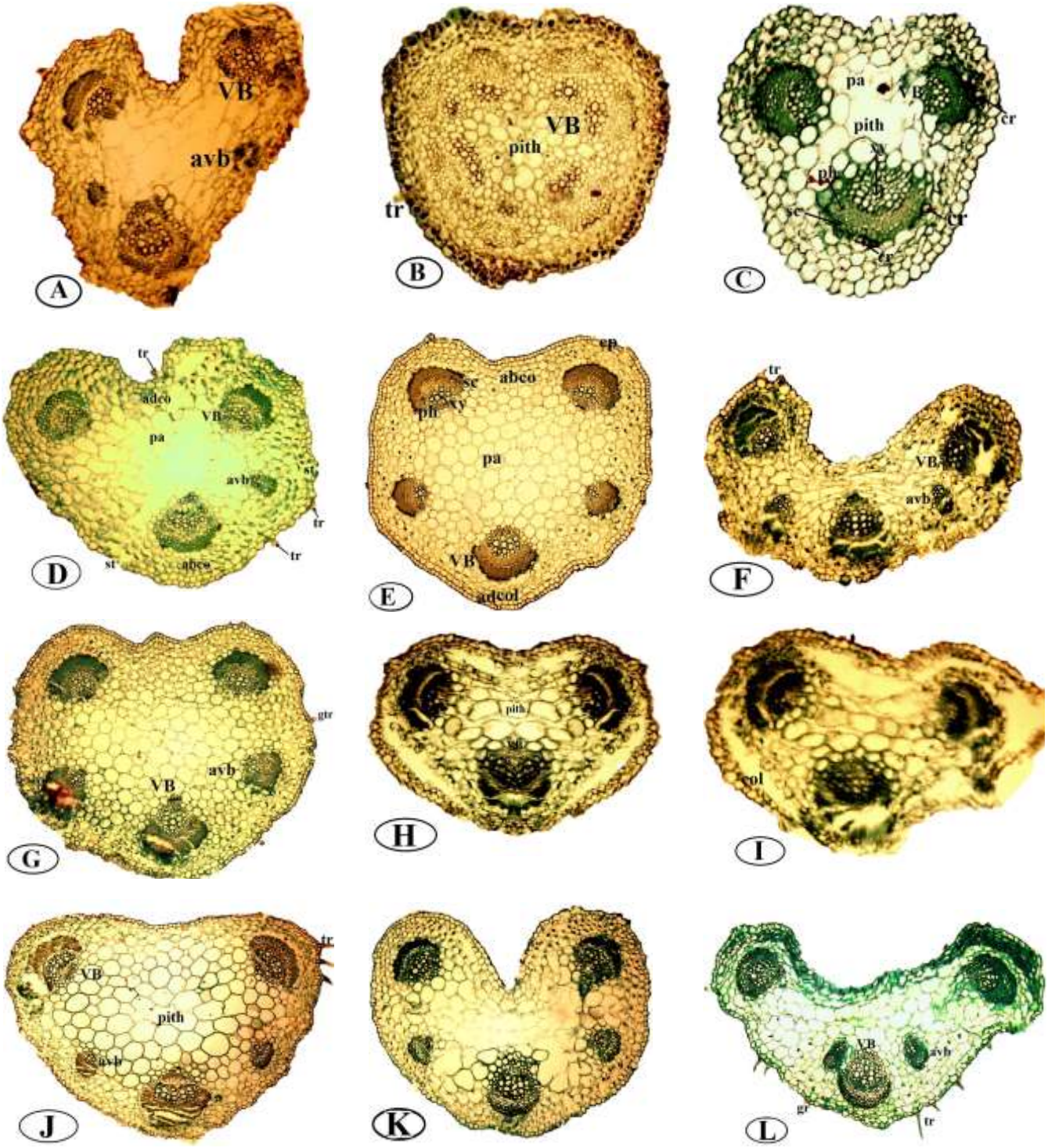

Fig 1. Variations of petiole cross-section shapes of studied *Trifolium* species, showing the number, position and size of vascular bundles (4X); A: *T. ambiguun*, B: *T. arvense*, C: *T. campestre*, D: *T. cherleri*, E: *T. dasyurum*, F: *T. echinatum*, G: *T. fragiferum*, H: *T. grandiflorum*, I: *T. guestii*, J: *T. hirtum*, K: *T. lappaceum*, L. *T. leucanthum*. adco: adaxial collenchyma, abco: abaxial collenchyma, ep: epidermis, st: stomata, gr: glandular trichome, tr: non-glandular trichome, VB: vascular bundle, avb: accessory vascular bundle, ph: phloem, xy: xylem, pa: parenchyma, sc: sclerenchyma, cr: crystal

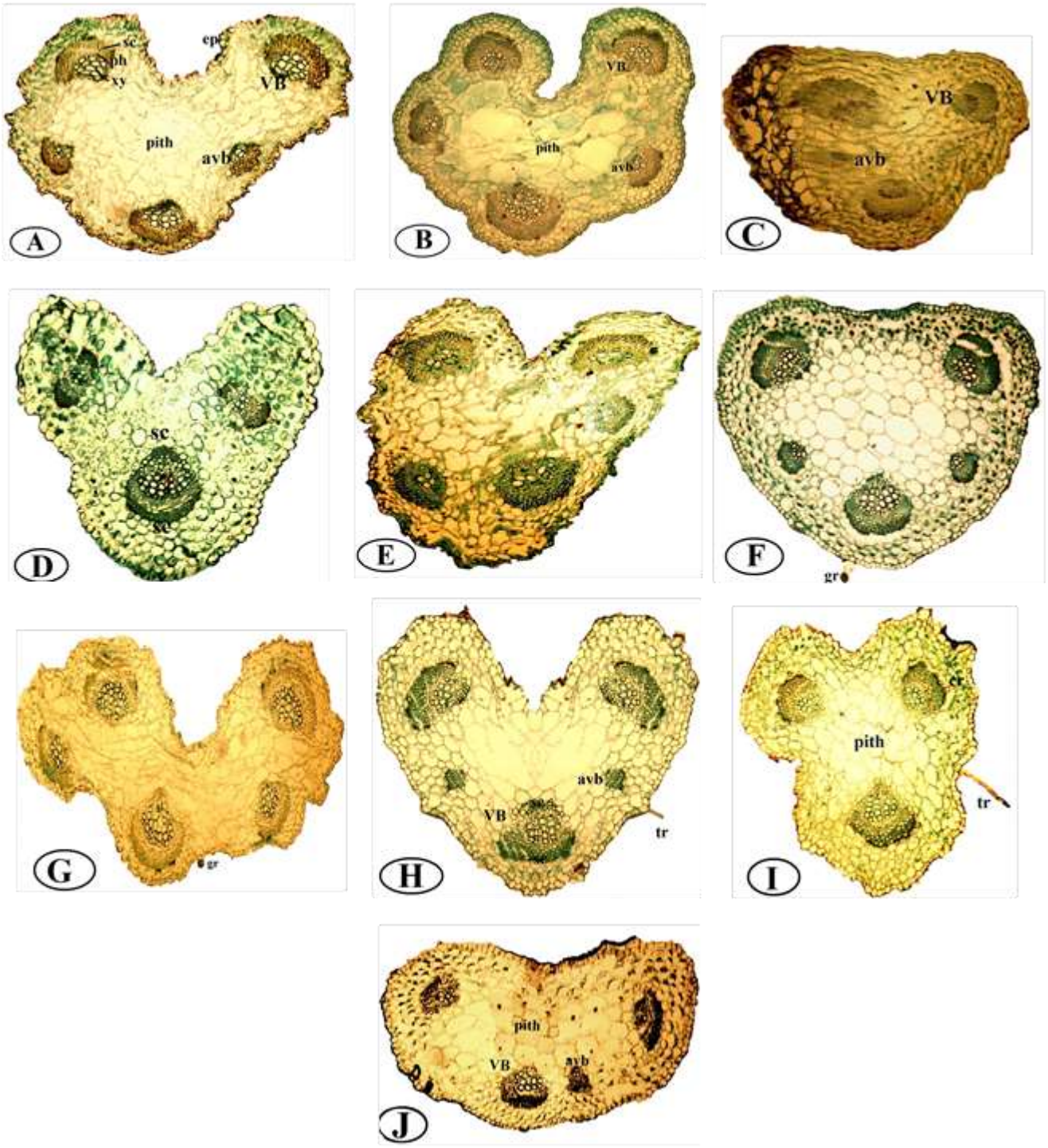

Fig 2. Variations of petiole cross-section shapes of studied *Trifolium* species, showing the number, position and size of vascular bundles (4X); A: *T. nigrescens subsp. nigrescens*, B: *T. physodes var glabrescens*, C: *T. pilulari*, D: *T. purpureum*, E: *T. resupinatum*, F: *T. scabrum*, G: *T. spumosum*, H: *T. stellatum* var. *stellatum*, I: *sylvaticum*, J: *T. tomentosum* var. *glabrescens*. col: collenchyma, ep: epidermis, st: stomata, gr: glandular trichome, tr: non-glandular trichome, VB: vascular bundle, avb: accessory vascular bundle, ph: phloem, xy: xylem, pa: parenchyma, sc: sclerenchyma, cr: crystal

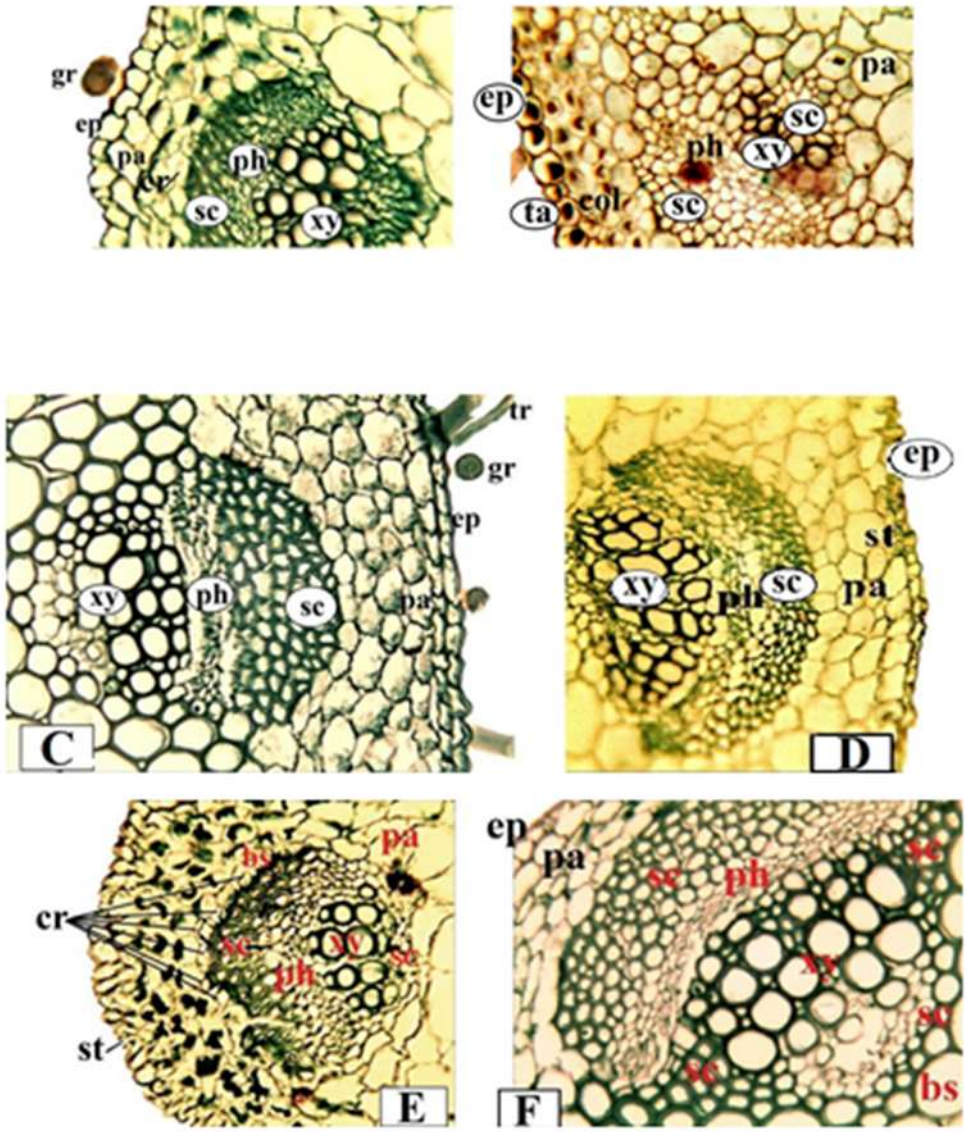

Fig 3. Cross-sections of petioles, all (40X). A: *T. scabrum*, presence of glandular trichomes, the vascular bundle completely surrounded with sclerenchyma. B: *T. arvense*, presence of non-glandular trichomes, sclerenchyma positions are above and under vascular bundle. C: *T. leucanthum*, presence of glandular and non-glandular trichomes. D: *T. ambiguum*, absence of trichomes, sclerenchyma position only above vascular bundle. E and F: Cross-sections of petiole vascular bundles E: *T. sylvaticum*, positions and distribution of crystals, and F: *T. stellatum* var. *stellatum*, the vascular bundle completely surrounded with sclerenchyma. ep: epidermis, gr: glandular trichome, tr: non-glandular trichome, ph: phloem, xy: xylem, pa: parenchyma, sc: sclerenchyma, col: collenchyma, st: stomata, cr: crystal, bs: bundle sheath.

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