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RESEARCH PAPER

Anatomical Comparison of the Stem and Leaf in Some of *Onobrychis* Mill. (Fabaceae) Species in Kurdistan region-Iraq

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A B S T R A C T:
In the current research, the anatomical characteristics of stem and leaves cross sections of 11 species belong to the genus *Onobrychis* Mill. have been studied. From this study, some anatomical features, such as the outline of stem and leaves cross sections are described, and the differences were determined among them. The epidermis trichomes, collenchyma, cortex, xylem, and phloem layers of the stems were measured toward the pith and there was a variation between the studied species. The upper and bottom epidermal layers were labeled on the leaf. The variations of midrib outlines, main vascular bundles shapes and their characters among the studied taxa microscopically have been determined.

KEY WORDS: Cross section, Midrib, *Onobrychis*, Outline, Paraffin, Stem, Taxonomy.
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1. INTRODUCTION:

The Fabaceae L. is one of the great dicot families, which consist of 730 genera and about 19500 species, it is important economically plants (Simpson, 2006). *Onobryehis* Miller considered one of the most difficult genus in the family, within the tribe Hedysareae of subfamily Faboideae. Yet, only 162 species were described in the genus (Boissier, 1872; Sirjaev, 1925a; Sirjaev, 1925b; Sirjaev, 1926; Bal1, 1968; Hayek, 1970; Hedge, 1970b; Mouterde, 1970; Schischkin and Bobrov, 1972a; Bobrov, 1972b; Townsend, 1974; Meikle, 1977; Rechinger, 1984; Tan and Sorger, 1986; Zohary, 1987; Davis *et al*., 1988; Duman and Vural, 1990; Aktoklu, 1995). The genus *Onobrychis* comprises about 170 species that densely distributed in Irano-Anatolian Caucasian, South West Asia and Mediterranean and in temperate Europe in the world (Cronquist, 1981; Zohary, 1987; Aktoklu, 2001).

There are 23 species of *Onobrychis* are recorded in the floramof Europaea (Ball, 1968), while the major center of distribution ranges from Central Asia. Ildis, (2005) confirmed 56 species, which are 27 endemic. The genus *Onobrychis* is restricted to the Palearctic region. Its distribution began from the Mediterranean region to Caucasia, the Zagros Mountains and central Asia. *Onobrychi* is concentrated in different places as in Iran 53 species, Caucasia 39 species, Anatolia 52 species, and only 8 species reach the Iberian Peninsula in the west. So, Irano-Anatolian region is regarded as one of the most prominent genetic diversity centers. The anatomical characteristics are considered as a piece of evidence that apply in the taxonomic study from a century ago (Radford,*et al*., 1974; Al-dabbagh and Saeed, 2019a). Stuessy, (1990) was interpreted the anatomical information is often quite helpful in solving taxonomical problems, and the anatomical data can help in the interpretation of evolution. Singh, (2010) stated

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that the anatomical characteristics are observed by using light microscope. The anatomy of some species of *Onobrychis* was investigated in an Atlas for trees, herbs, and shrubs (Schweingruber *et al.*, 2011).

Many studies conducted on the genus *Onobrychis* in Iran morphological, palynological and karyological traits were employed to estimate the interspecific relationships ((Karamian *et al*., 2009, 2010; Ranjbar *et al.*, 2009, 2010b. However, there is no report on anatomy of the genus. Anatomical characters are not always as useful as morphological characters for plant identifications. However, there is no record on anatomy of the *Onobrychis*. Anatomical characters are not constantly as useful as morphological characters for identifications of plant species. However, they are well-established criteria and can offer significant assistance in plant taxonomy (Güvenç & Duman, 2010; Ranjbar *et al*., 2010a; Güvenç *et al*, 2011). Moreover, genetic diversity in a worldwide collection on *Onobrychis* using anatomical, molecular and morphological was studied by Zarrabian *et al*, (2013).

Particularly, the genus Onobryehis, that covered by this study in Kurdistan Region-Iraq and to enhance flora of Iraq. The aim of the present study was focused on anatomical characters on some perennial Onobrychis species of Fabaceae family in Kurdistan region-Iraq for the first time.

2. MATERIALS AND METHODS

Samples of *Onobrychis* were prepared from herbarium specimens. In addition, to those were collected specimens were stored in the herbarium of the Department of Biology/ Sciences College/ Salahaddin University/ Erbil/Iraq.

For the anatomical study, herbarium stems, and leaves put in the 1% NaOH for overnight, then transfer to fixed solution as FAA (Formalin-Acetic acid- Alcohol) for 24hr. After that the samples were dehydrated by using series concentrations of ethyl alcohol (80%, 90%, 95%, 100%) for 3-4-1hrs for each concentration, after that the samples were placed in xylene combinations (twice time) for 3-4hrs. for each time for clearing processes. Then infiltrating the samples by transfers them in paraffin and left in the oven at 60^{O}C for a night.

The blocks of paraffin were made by using a suitable manner for cutting. Slide sections have been cut with the thickness of 8-10 micrometer using the rotary microtome (Bright, LTD), the ribbons placed and mounted on slides carefully, then transferred to a hot plate for overnight. The slides were stained by safranin and fast-green, and covered by coverslips after adding a drop of DPX. The prepared slides examined and imaged by Light Microscopes (Olympus AC100 with a camera, Japanese-made) (Najmaddin and Mahmood, 2016); (Najmaddin, 2019).

3. RESULTS

3.1. General anatomical description of stems

The results showed that were different outline shapes of the stems in studied *onobrychis* species; the stem outline cross sections in studied taxa are; triangular in *O. caput-galli* Lam , ovate in *O. aequidentata* Sm. and *O. schahuensis* Bornm, semi-circular in *O. crista-galli* Lam, *O. haussknechtii* Boiss., *O. megataphros* Boiss , and *O. acaulis* Bornm., circular in *O. Susiana* Nab., polygonal in *O. kotschyana* Feuzl., *Rectangular in O. ptolemaica* Delile, and *O. galegifoliaa* Boiss (Figure 1).

The epidermis consists of a single layer in all studied taxa; the cells are triangle sub–circular to sub rectangular in shape. The epidermis of most species compasses trichomes except *O. caput-galli, O. aequidentata*, and *O. megataphros*. The multicellular glandular hairs are observed in *O. cristagalli* and *O. schahuensis*, while the multicellular non- glandular hairs were observed only in *O. haussknechtii*. Nevertheless, the non-glandular and glandular multicellular trichromes are observed in the rest species (Figure 2 and Figure 3).

Beneath the epidermis there is a continuous ring of 12-13 cell thinned of collenchymatous cells that are interrupted by parenchyma tissue of cortex, the differences of row numbers in conformity with the thickness of the spaces between epidermis and pericycle. They

appear peltate of 3-7 cell thickness in *O. caput-galli*; 5-9 cell thickness in *O. aequidentata*; 7-9 cell thickness in *O. haussknechtii*; *O. megataphros* and *O. kotschyana*; 3-4 cell thickness in *O. susiana*; 5-8 cell thickness in *O. ptolemaica*; 3-7 cell thickness in *O. acaulis* and *O. galegifoliaa*, laqunar of 6-7 cell thickness in *O. cristagalli*, and angular of 9-12 cell thickness in *O. schahuensis*.

The prismatic crystals and tannins are observed stems of all taxa, whereas the secretory cells observed only in cortex of *O. schahuensis* stem. Cup-shaped 13-24 cell thickness of fibers are arranged in tangential layers and are located above phloem of each vascular bundles, surrounded by parateacheal and marginal parenchyma.

Opened vascular bundles are arranged in continuous close ring-like inward surrounding the pith, there were two groups are separated according to the vascular bundles size, first the uniform sizes as in *O. caput-galli*, *O. aequidentata*, *O. cristagalli*, *O. haussknechtii*, *O. megataphros*, *O. susiana*, *O. kotschyana*., and *O. galegifoliaa*, and the second the vascular bundles are vary in sizes such as *O. schahuensis*, *O. ptolemaica*, and *O. acaulis*. The pith is innermost of the stem toward the center is a mass of parenchymatous tissue with different sizes of thin wall, with large cell spaces parenchyma cells, which almost become hollow at maturity.

3.2. General anatomical description of leaves

The leaves are consisting of a single layered in all examined taxa. Upper epidermis composed of rectangular, oval, square or sometimes irregular shaped cells in *O. galegifoliaa*, while rectangular, occasionally square or irregular shaped cells in *O. megataphros* and *O. ptolemaica*, but oval, rectangular sometimes irregular shaped cells in *O. schahuensis*. It is depressed square or occasionally irregular shaped cells in *O. megataphros*, *O. acaulis*, *O. aequidentata* and *O. caput-galli*, it is depressed rectangular oval shaped cells in *O. kotschyana*, *O. susiana*, and oval, rectangular occasionally regular shaped cells in *O. haussknechtii* (Figure 4).

The trichomes were noticed in both epidermis in most studied taxa except *O. caput-galli*, *O. aequidentata*, and *O. crista-galli*. There are non-glandular unicellular trichomes are noticed in *O. kotschyana*, *O. megataphros*, *O. galegifoliaa*, *O. haussknechtii*, *O. acaulesent*, *O. schahuensis* and *O. susiana*. Whereas the non-glandular multicellular trichomes are observe in *O. kotschyana* , *O. susiana*, *O. galegifoliaa* and *O. ptolemaica*. Sessile unicellular-glandular hairs observe in *O. susiana*, *O. haussknechtii*, and *O. schahuensis*. In addition, the glandular multicellular trichomes are present in *O. kotschyana* , *O. susiana*, *O. haussknechtii*, and *O. megataphros*. Nevertheless, the *O. haussknechtii* was unique in possessing the non-glandular branched multicellular trichomes (Figure 4).

The leaves are amphistomatic, and the mesophyll is isobilateral in all examined taxa, whilst, they differ in the length, size, distribution and abundance. Underneath of upper epidermis, there are palisade parenchyma cells. Palisade parenchyma is 3–5 layers and cells are rectangular or cylindrical oval shaped in *Onobrychis acaulis*, *Onobrychis aequidentata* and *Onobrychis caput-galli*. Palisade are arranged in 4–6 layers of cells and sometimes rectangular oval or irregular shaped in *O. haussknechtii*, *O. megataphros*, *O. schahuensis*, *O. kotschyana* and *O. ptolemaica*. Whilst arranged in 5–6 layers cells and are usually cylindrical or irregular shaped in *O. cristagalli*, *O. galegifoliaa* and *O. susiana* (Figure 4).

The margin outlines of leaves showed different outline shapes in studied *Onobrychis* species, the margin is straight rounded as in *O. caput-galli*, *O. cristagalli*, *O. schahuensis*, *O. susiana*, *O. ptolemaica*, *O. acaulis* and *O. galegifoliaa*. While rounded upward in *O. aequidentata*, *O. megataphros*, *O. kotschyana* . When rounded slightly downward in *O. haussknechtii* (Figure 5).

The midrib outlines of studied species are various in shapes, the midrib outline is approximately eminent in abaxial, and convex in adaxial as in *O. caput-galli*, while the abaxial is

rounded and concave in adaxial such as *O. aequidentata* and *O. galegifoliaa*. In *O. haussknechtii, O. kotschyana* and *O. megataphros* the abaxial is rounded and straight in adaxial. The midrib among studied taxa, which is V-shaped, has been observed in *O. cristagalli.* In *O. schahuensis* the abaxial is U-shaped and convex in adaxial, whilst in *O. susiana* is convex in abaxial and adaxial. In *O. ptolemaica*, elongate rounded in abaxial and straight in adaxial, however, the midrib in *O. acaulis* is straight in adaxial and semi-circle dish-shaped in abaxial (Figure 6).

Midribs main vascular bundles are single closed in all studied taxa, surrounded by a single layer of parenchymatous bundle sheath. But they are differing in size and shapes, the vascular bundle is small, circular, ovate shape in *O. caput-galli, O. cristagalli, O. haussknechtii* and *O. kotschyana* , while large, circular, ovate shape in *O. susiana* and *O. ptolemaica.* Small semi-circular crescent shaped in *O. megataphros*, the rest are crescent semi-circular in the rest such as *O. aequidentata, O. schahuensis, O. acaulis* and *O. galegifoliaa* (Figure 6).

4. DISCUSSION

The epidermis comprise of a single layer in all studied taxa, the cells are triangle, sub–circular to sub-rectangular, ring-shaped, polygonal, or elliptic with a reticular or smooth border in shape (Butnariu, *et al*, 2005; 2006, Rodino, *et al*, 2014). The lamina mesophyll of leaves in all studied of *Onobrychis* species are heterogeneous. The prismatic crystals, secretory cells with tannins are observed in all taxa, as has been reported by (Shweingruber *et al*., 2011). *Onobrychis* represents a triangle shape in stem outlines. The palisade cells are oblong in shape, and the spongy cells have different irregular shapes with large intercellular spaces, the palisade and spongy cells are arranged in to 8-9 layers depended on the location and lamina thicknesses as mentioned by (Al-dabbagh and Saeed, 2019b). The cortex is consists of 4 layers of semi–circular to sub-rectangular parenchymatous tissue (Butnariu and Giuchici, 2011; Ianculov, *et al*., 2004; Butu, *et al*., 2014).

Onobrychis aequidentata, *Onobrychis cristagalli* and *Onobrychis haussknechtii* shows elliptic shape in stem outlines. The stem outlines in cross sections are circular or circular to elongate, they relatively have a similar shape in four species *Onobrychis megataphros, Onobrychis schahuensis, O. susiana, and O. kotschyana* . Whereas, *O. ptolemaica, O. acaulis,* and *Onobrychis galegifoliaa* have hexagonal shaped stem outlines covered with hairs reticular or smooth on their surface.

The tannins and crystals present in parenchyma, vascular bundle and pith of species. Tannin and prismatic crystals are cited by various authors for the family. The collenchymatous (lacunar collenchyma), consists of 2-6 layers of collenchyma tissue, the differences of row numbers in conformity with the thickness of the spaces between epidermis and pericycle.

The endodermis is 1-6 layers of large well-developed parenchyma cells, which the numbers of row are depended on the endodermis thicknesses and conversely with numbers of hypodermal cells.

This study investigated that the trichomes are unicellular glandular, non-glandular, and sessile multicellular glandular and non-glandular in the stem of the genus taxa. However, they are vary in dense and abundance in addition to their size, length. Obviously, the multicellular glandular trichomes are terminating a head which is either globose or clavate, besides, the trichomes walls are impregnated with plentiful crystalline granules. These glandular trichomes usually excrete an extremely sticky substance (Struwig *et al*., 2011).

Under upper epidermis, between palisade parenchyma cells there are voluminous cylindrical shaped secretory cells in all examined taxa as confirmed by (Najmaddin and Khalid, 2017). The secretory cells are presence in all examined taxa.

Spongy parenchyma cells are cylindrical, oval or circular shaped in all taxa Spongy parenchyma consists of (2-5) layered in all species. Crystalliferous cells frequently form a sheath along the outer boundary of the pericyclic sclerenchyma (Metcalfe and Chalk, 1950). Solitary prismatic crystals are recognized to be distributed in the cortex, rarely in the phloem and xylem. (Bakr and Fthulla, 2017). These Tanins cells are circular or oval or occasionally irregular shaped present in the cortex, palisade parenchyma and spongy parenchyma and vascular bundle cells. Midrib outline showed different shapes and different vascular size, these variations might have taxonomical value (Al-dabbagh and Saeed, 2019b).

5. CONCLUSION

In this investigate concluded the species differ in the outline shapes of the stem, leaves midrib and margin. Prismatic crystals, fibers, and tannins are found in stems present. in stems of all studied taxa. Trichomes are presented which are multicellular or unicellular and glandular or non-glandular. The leaf mesophyll has no such great variation of taxonomic value.

Conflict of Interest (1)

There is no conflict of Interest.

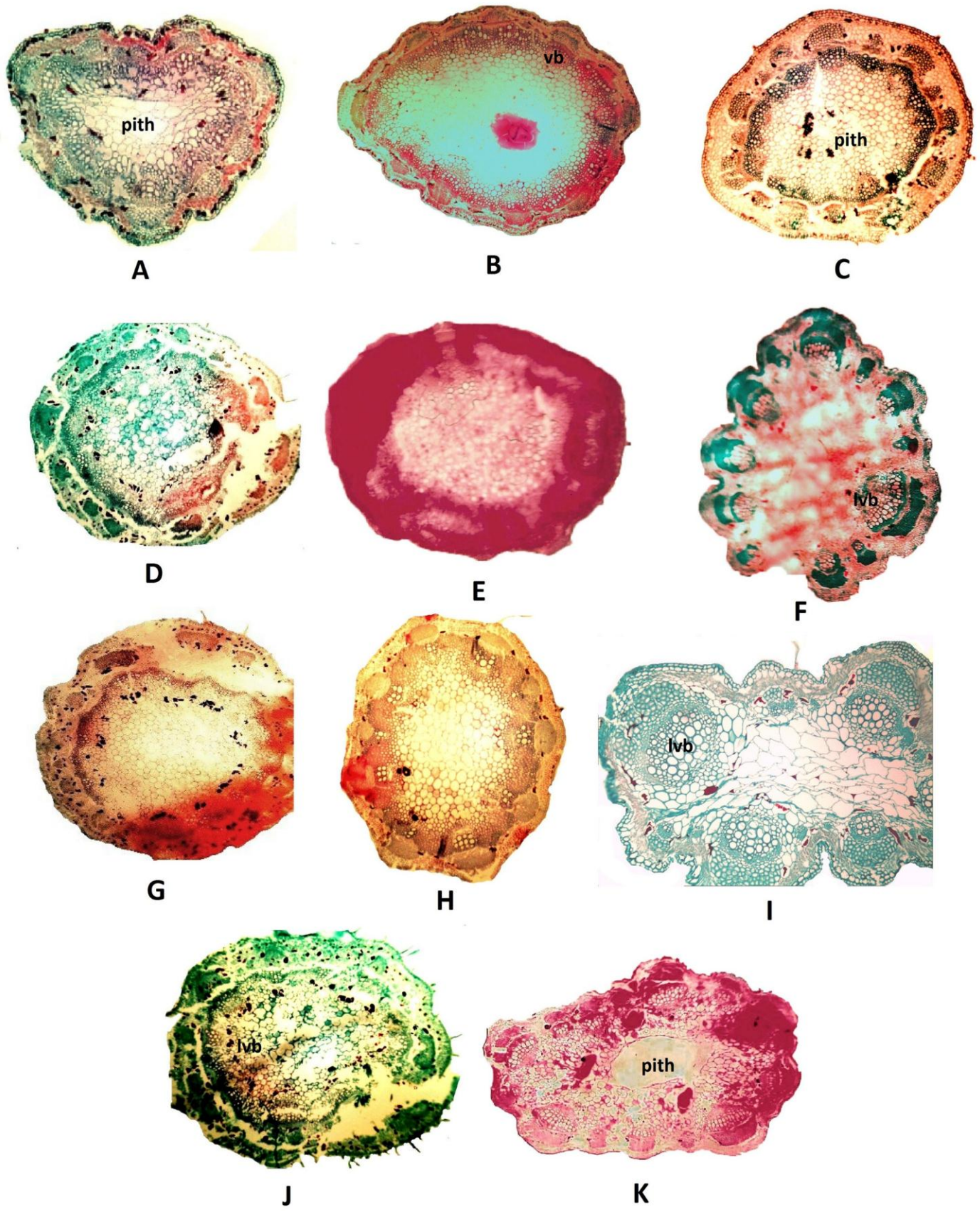

Fig. 1. Variations of stem cross section outlines of the studied *Onobrychis* Species, A- *O. caput-galli*, B- *O. aequidentata*, C- *O. cristagalli*, D- *O. haussknechtii* , E- *O. megataphros*, F- *O. schahuensis*, G- *O. susiana*, H- *O. kotschyana*. I- *O. ptolemaica*, J-*O. acaulis*, K- *O. galegifoliaa* (4X).
vb= vascular bundle, lvb=large vascular bundle

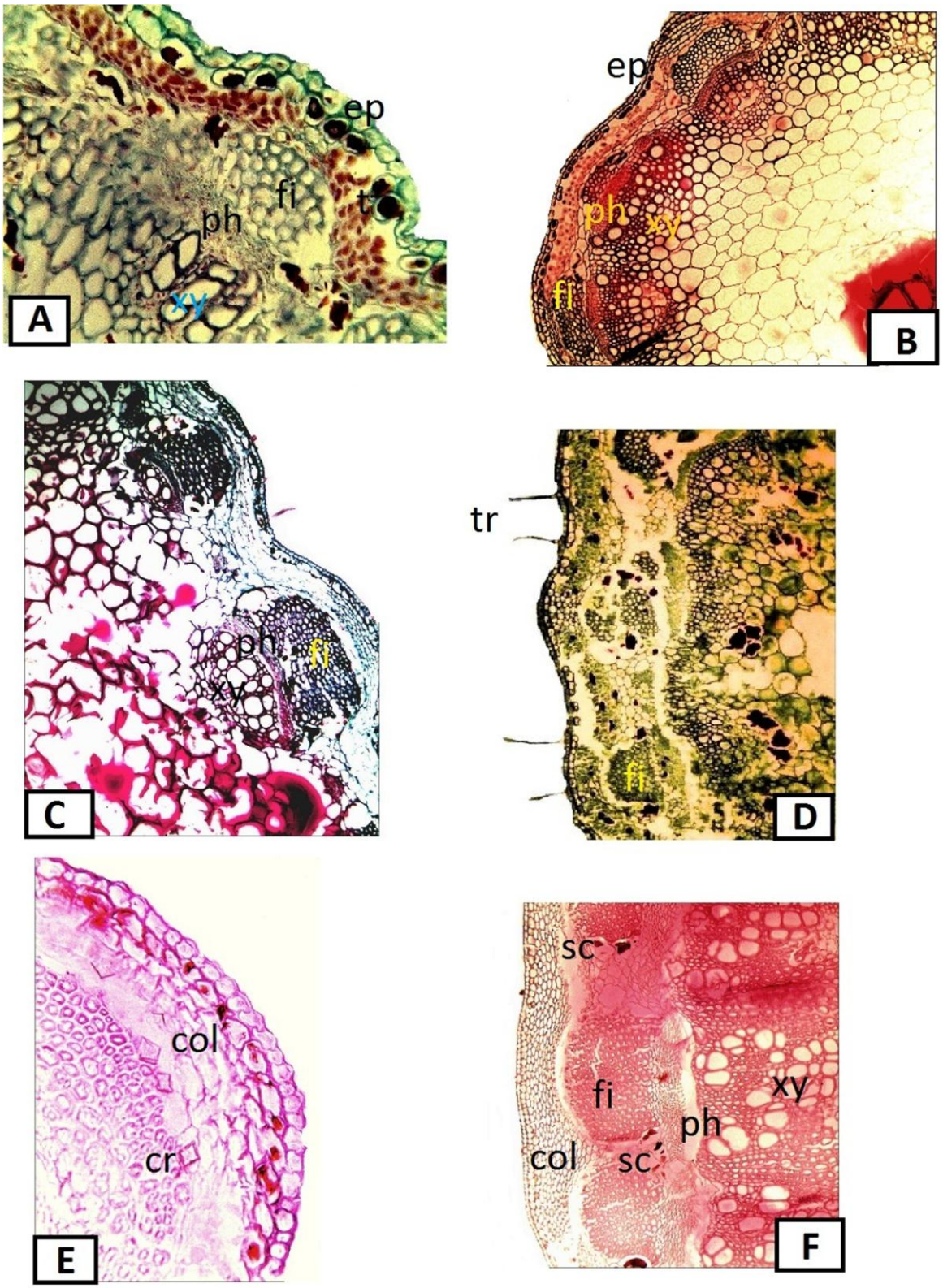

Fig. 2. Variations of stem cross sections of the studied *Onobrychis* Species, A- *O. caput-galli*, B- *O. aequidentata*, C- *O. cristagalli*, D- *O. haussknechtii* , E- *O. megataphros*, F- *O. schahuensis* (40X).
ep= epidermis, t= tannin, col= collenchyma, fi= fiber, ph= phloem, xy= xylem, cr= crystal, sc= secretory cells, tr= trichome.

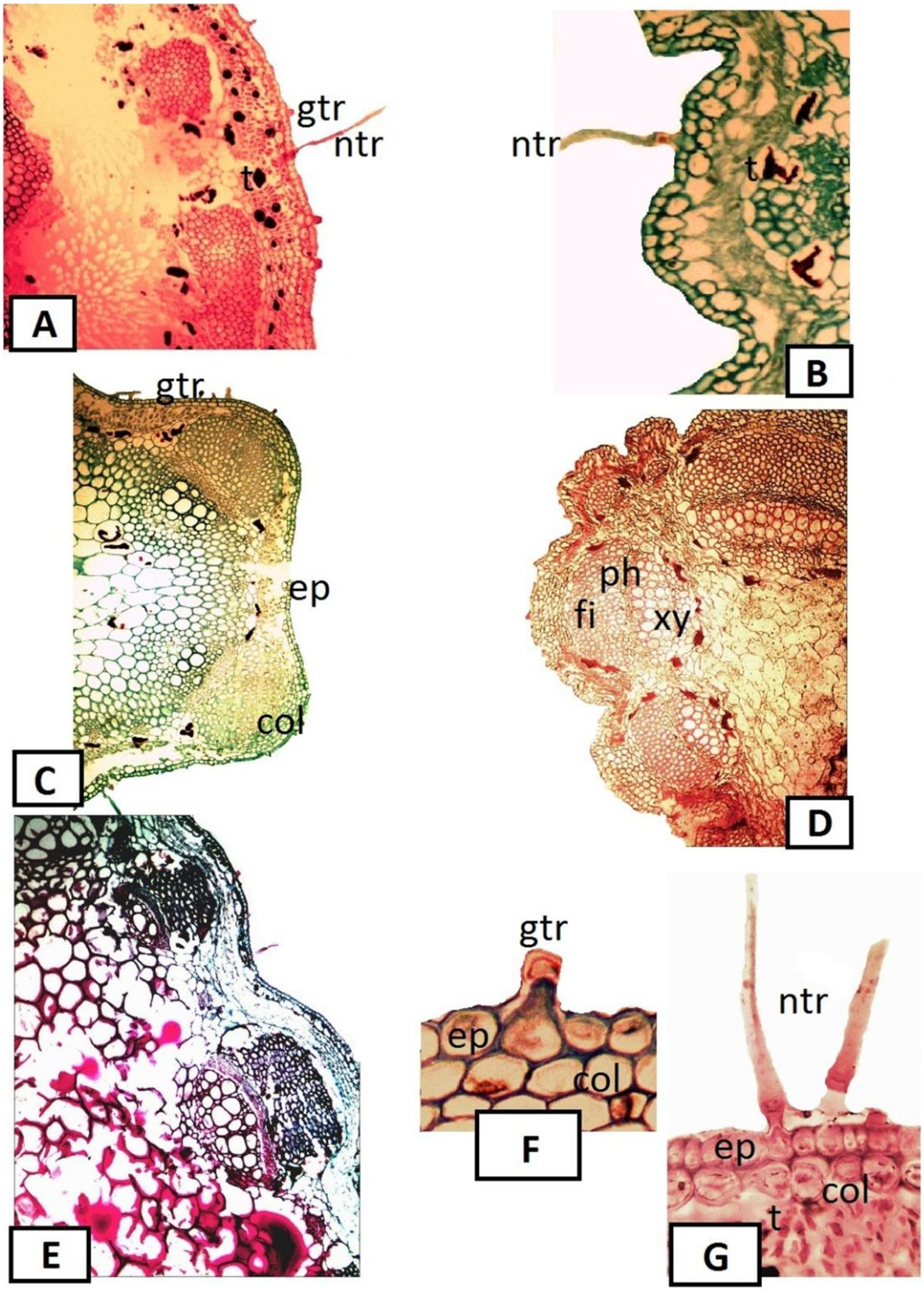

Fig. 3. Variations of stem cross sections of the studied *Onobrychis* Species, (A-E); A- *O.susiana*, B- *O. kotschyana*. C- *O. ptolemaica*, D-*O. acaulis*, E- *O. galegifoliaa*; and (F-G) trichomes, F- glandular hair, G- non-glandular hairs (40X).
ep= epidermis, col= collenchyma, fi= fiber, ph= phloem, xy= xylem, cr= crystal, t= tannin, sc= secretory cells, gtr= glandular trichome, ntr= non-glandular trichomes

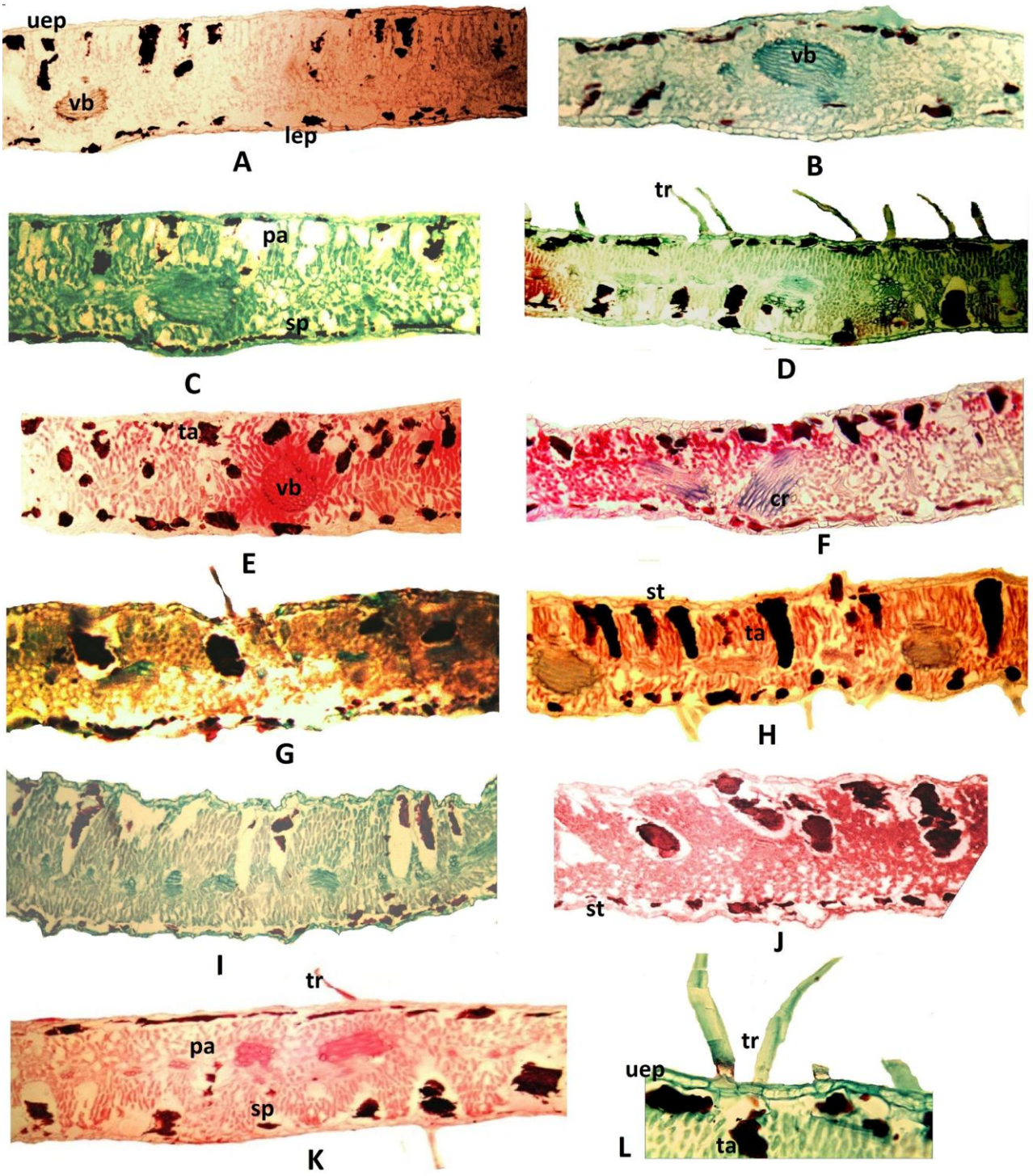

Fig. 4. Variations of lamina cross sections of the studied *Onobrychis* Species, A- *O. caput-galli*, B- *O. aequidentata*, C- *O. cristagalli*, D- *O. haussknechtii* , E- *O. megataphros*, F- *O. schahuensis*, G- *O. susiana*, H- *O. kotschyana*. I- *O. ptolemaica*, J-*O. acaulis*, K- *O. galegifoliaa* (4X) and L- *O. haussknechtii*, magnified portion of lamina(40X).

Uep= upper epidermis, lep= lower epidermis, pa= palsied, sp= spongy cells, st= stomata, vb= vascular bundle, ta= tannins, tr= trichomes, cr= crystal.

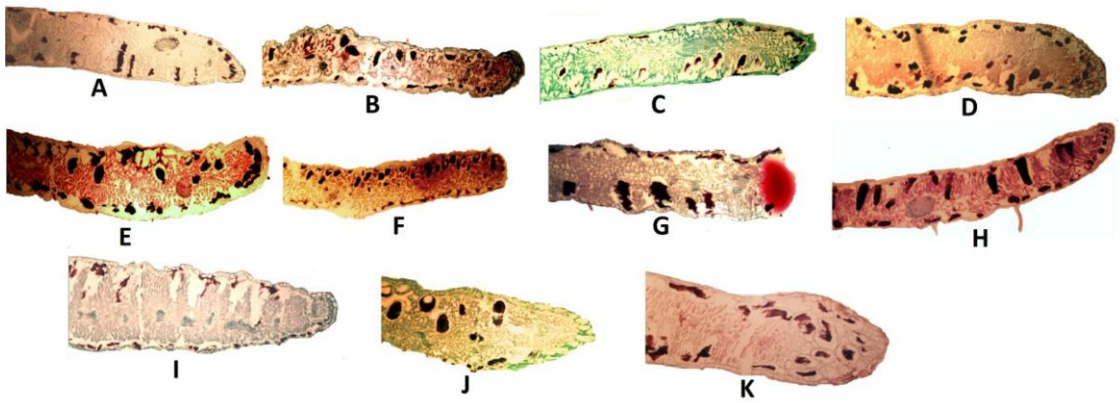

Fig. 5. Variations of leaves margin cross sections of the studied *Onobrychis* Species, A- *O. caput-galli*, B- *O. aequidentata*, C- *O. cristagalli*, D- *O. haussknechtii* , E- *O. megataphros*, F- *O. schahuensis*, G- *O. susiana*, H- *O. kotschyana*. I- *O. ptolemaica*, J-*O. acaulis*, K- *O. galegifoliaa* (4X).

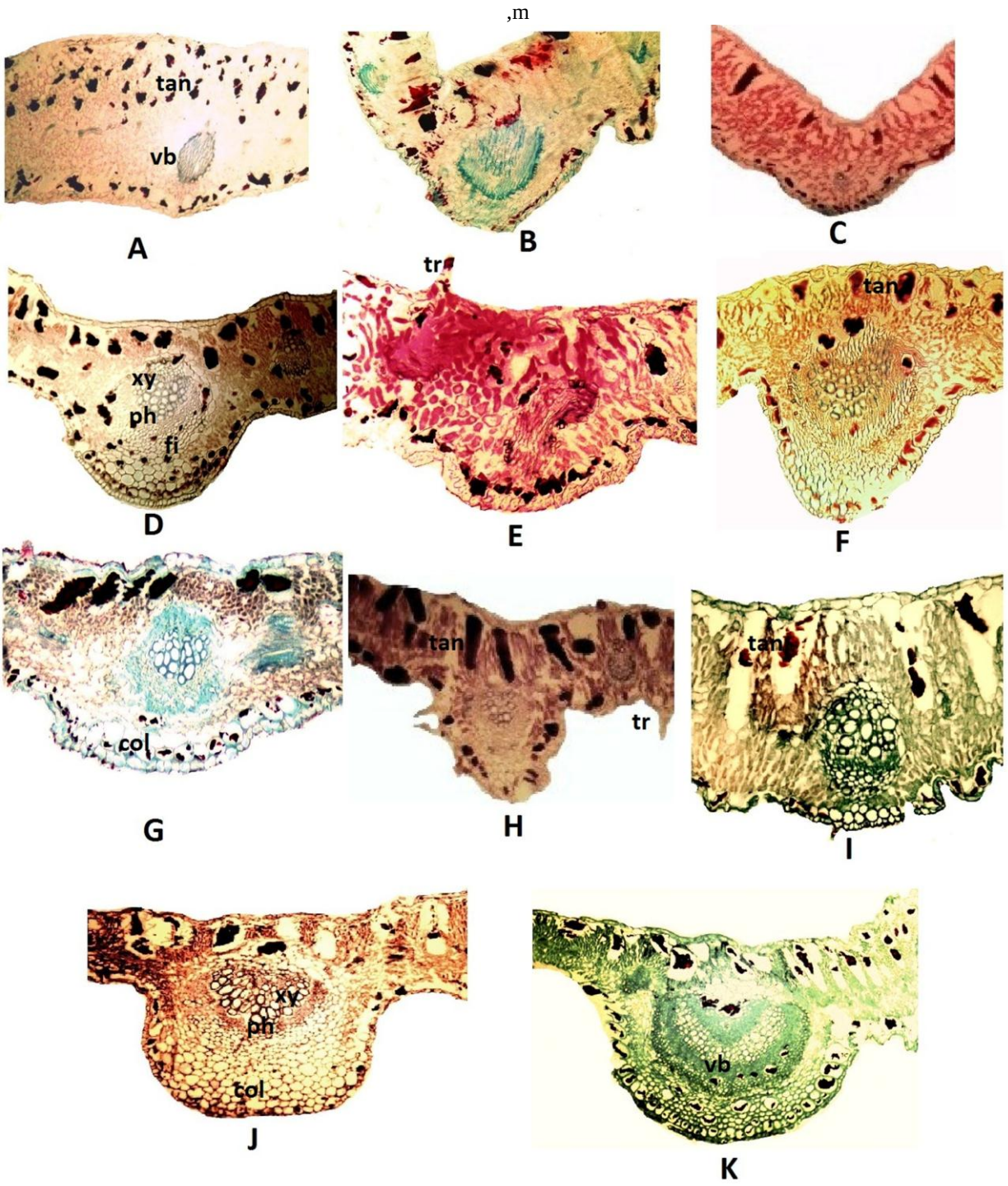

Fig. 6. Variations of midrib cross section outlines of the studied *Onobrychis* Species, A- *O. caput-galli*, B- *O. aequidentata*, C- *O. cristagalli*, D- *O. haussknechtii* , E- *O. megataphros*, F- *O. schahuensis*, G- *O. susiana* H- *O. kotschyana*. I- *O. ptolemaica*, J-*O. acaulis*, K- *O. galegifoliaa* (10X).
vb= vascular bundle, ta= tannins, ph= phloem, xy= xylem, tr= trichome, cl= collenchyma.

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