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Research article

Vegetative and reproductive anatomy of *Vigna radiata* L.

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Abstract: In this study, anatomical features of the stem, petiole, leaf and flower of *Vigna radiata* L. (ML2017 Genotype) belonging to Fabaceae family (Subfamily Papilionoideae) were examined. Basic structure of a dicotyledonous plant is showed in stem and petiole. Their transverse section consists of: epidermal and collenchyma layers, cortical layer (parenchyma cells and pericyclic fiber) and stele (vascular bundles, secretory cells and pith); however there are differences in shape and position of vascular bundles. In the stem, this bundles located on a continuous ring but in the petiole are cutting and divided into two large adaxial and three abaxial bundles forming main folaire trace, above which lie laterally a pair of secondary bundles. In the leaf is important the number of mesophyll palisadic and spongy layers, stomatal type (paracytic) and stomatal density (48.3%). The secretory cells are in the stem, petiole and leaf. The flower structure is pantamerous with 5 sepals, 5 petals (standard, wings and keel) androecium is of diadelphous and gynocium one carpel and ovary one locule with marginal placentation. In general anatomical charecteristics are very important and could be used in diagnostic key of taxa at all taxonomic levels.

Keywords: Anatomy - Stem - Petiole - Leaf - Flower - *Vigna radiata* - Fabaceae.

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INTRODUCTION

The green gram, *Vigna radiata* L. of Fabaceae family (subfamily Papilionoideae) is one of important pulse crops in Iran, India, China, Japan, American and Vietnam. It is a protein rich staple food. It contains about 25 percent protein. It supplies protein requirement of vegetarian population of the country. This is consumed in the form of split pulse as well as whole pulse, which is an important source of human food and animal feed, Green gram also plays an important role in substaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen (Mbagwu & Endeoga 2006, Singh *et al*. 1997). The economic importance *Vigna* species exhibits a good grow successfully in extreme environments such as high temperature, low rain fall and pore soils with few economic inputs (White 1983). Some anatomical and morphological features of the subfamily Papilionoideae were reported by Webster & Cardina (2004). The variations in polar unit symmetry and differences in wall sculpture of pollen grains have been used by many authors in the delimitation of various taxa (Agwu & Uwakwe 1992, El-Ghamery 2003). Metcalfe & Chalk (1950) mentioned that the stems of *Lathyrus* and *Vicia faba* (Fabaceae) have special features. Aim of the work was to provide details stem,petiole, leaf and flower anatomical of *Vigna radiata* L. (ML2017 Genotype) for the first time and give a comprehensive anatomical description of it aerial parts.

MATERIALS AND METHODS

The plant samples were collected from Ramhormoz Eslamic Azad University research field (IRAN). The for cross sections, 2 cm slices from stem, petiole and leaf were chosen and softened in a mixture of

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glycerine/ethanol 70% (0.5:0.5) for one week. Cross sections were made with using commercial razor blades. The sections were stained with methyl blue (0.5 g methyl blue + 1000 cc H_2O) and carmine (4 g KAl (So_4) + 100 cc H_2O + 1 g carmine and boiled for 20 min) and mounted on the slides using Canada balsam. Then they were photographed with a digital photo-camera attached to light microscope at 10-40X magnifications. Anatomical characters, which were selected, included outline shape of the cross section, the shape of epidermal cells, surface trichomes, the number of collenchyma layers, the cambium of vascular bundles, the number of pericyclic fiber layers, the shape of parenchymatous cells in pith, stomatal type, mesophyll position, stomatal density, the number of petiole vascular bundles, petiole shape, the secretory cells (Fahn 1990, Hasan & Heneidak 2006, Mehrabian *et al*. 2007).

For microtomicsections, flowers collected fixed in absolute FAA (2 cc Formalin 37% + 17 cc ethanol 96% + 0.6–1.0 cc Acetic acid) washed with fresh water, then they were dehydrated in ethanol series with ulterior toluene infiltration and embedding in paraffin wax. Sections were cut at 8–12 mm with a rotary microtome. The slides were stained in oozine-hematoxilin and mounted in Canada balsam for light microscopy observation. The flower and anther parts were observed. Pollen grain morphology is showed with SEM, for this work pollen grains stabilized on aluminium stoks and coated with a thin layer of gold using coating equipments. Then, they were studied using Scanning Electron Microscope at central laboratory of Ahwaz Shahid Chamran University.

RESULTS

Stem anatomy

Stem anatomical features of the examined sample at the age of six weeks based on transverse sections of stem are shown in figure 1. The stem transverse section is ribbed. The epidermis layer consists of a single row of rectangular cells that covered with thin cuticle. The buliform cells are in this layer. These cells are big and vacuolar. There are single cell trichomes on some of epidermal cells. The circular collenchyma cellsin the row four is located very close to the epidermis. The cortex consists of parenchymatous oval cells with thin walls. The pericyclic cells show their transformation into fibers and one layer of fibrous strands are developed. The stele consists of collateral vascular bundles arranged in a ring that separated from one another by interfascicular cambium. In per bundle are intrafascicular cambium layers six among of xylem and phloem. Xylem is composed of protoxylem toward the plant center and metaxylem to the side of plant apical. The secretory cells are located very close to the phloem that these cells have Glocusidas material. The bundles are relatively different in size and number. There are six large bundles located opposite ridges. There is also large pith in the stem center and consists of polygonal parenchymatous cells which tend to decrease in size towards the periphery small triangular intercellular spaces are visible.

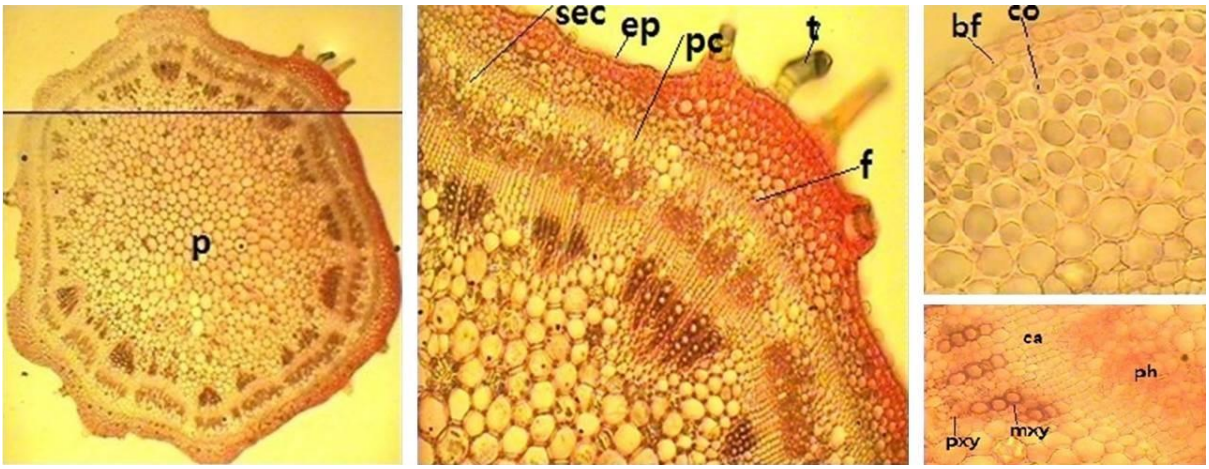

Figure 1. Stem transverse section of *Vigna radiata* L., ML2017 genotype (objective X_{10}, X_{40}). [p: Pith, sec: Secretory cell, ep: Epidermis, pc: Cortex parenchyma, t: Trichome, f: Pericyclic fiber, bf: Buliform, co: Collenchyma, ca: Cambium, ph: Phloem, pxy: Protoxylem, mxy: Metaxylem]

Petiole anatomy

Transverse section taken from the petiole of sample showed the following features (Fig. 2). The petiole has irregular shape and consists of: The epidermal cells is also uniseriate with rectangular shaped cells and covered

with simple, unicellular and unbranched trichomes. One layer of circular collenchyma cells is located under the epidermis. The cortex consists of orbicular parenchymatous cells. The stele is clearly divided into large two adaxial bundles and smaller three abaxial bundles forming main trace, above which lie laterally a pair of secondary bundles. Pericyclic fibers two layer are present as a separate layer above the phloem of each bundle of the main folaire trace only (adaxial and abaxial bundles) while each secondary bundle has its own separate fiber cap. Petiole vascular bundles is collateral type such as stem. The secretory cells is close of phloem. The pith is composed of polygonal parenchymatous cells with intercellular space.

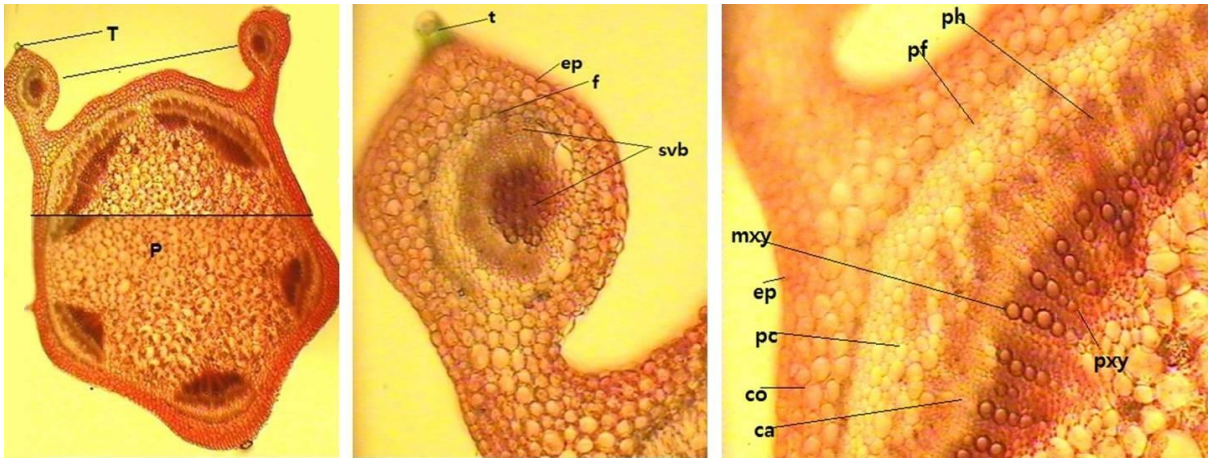

Figure 2. Petiole transverse section (objective X_{10}, X_{40}). [T: Trichome, p: Pith, ep: Epidermis, f: Fiber, svb: Secondary vascular bundles, ph: Phloem, pf: Pericyclic fiber, mxy: Metaxylem, pc: Cortex parenchyma, co: Collenchyma, ca: Cambium, pxy: Protoxylem]

Leaf anatomy

The upper and lower leaf epidermis layers are composed of uniseriate with rectungicular cells and buliform. In this layer are stomata that consists of guard cells typically kidney-shaped and ostiole and are located on the same level relative to the epidermal cells. The type of stomata observed is paracytic (Rubiaceae) and they occur on the surface of both sides being more abundant on the lower surface. The epidermal cells shape is angular polygonal. Stomatal density is 48.3 percent. The mesophyll is heterogenous and is composed of four layers of palisade cells and three layers of spongy cells. The midrib is well developed. The xylem and phloem are collateral; the xylem (one arches) is towards the upper side, while the phloems on the lower side. In the secondary rib are spiral vessels.

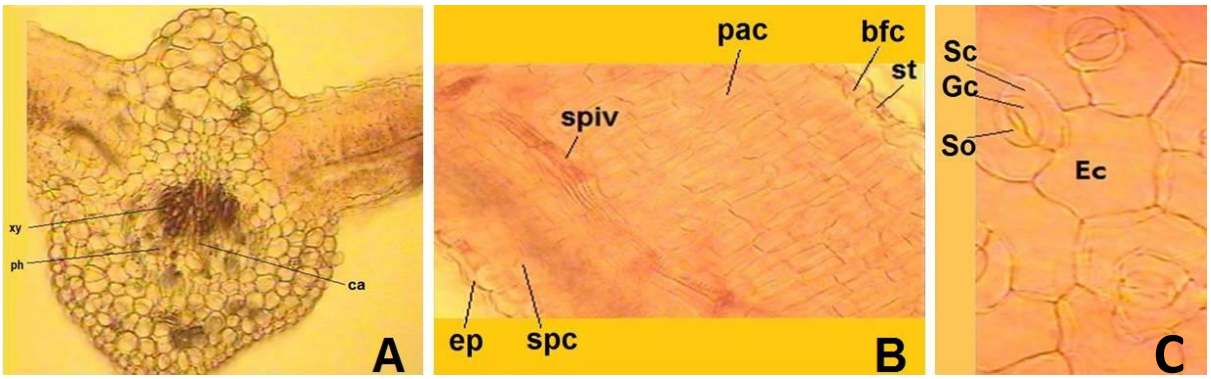

Figure 3. Leaf transverse section (objective X_{10}, X_{40}): **A**, Midrib; **B**, Blade; **C**, Paracytic stomata. [xy: Xylem, ph: Phloem, ca: Cambium, pac: Palisadic cell, bfc: Buliform cell, st: Stomata, spiv: Spiral vessel, ep: Epidermis, spc: Spongy cell, Sc: Subsidary cell, Gc: Guard cell, So: Stomataostiole, Ec: Epidemis cell]

Floral bud anatomy

Floral bud transverse section of *Vigna radiata* L. (ML2017 Genotype) plant is shown in figure 4. It is clear that sepals of calyxs are united and consists of two epidermal layers and 3–4 layers of ground tissue in between. The corolla is papilionaceous with one posterior (the standard or vexillur), two lateral petals (the wings) and two lower united anterior petals (the keel). The stamens are ten; each stamen consists of a two-lobed tetrasporangiate

anther born on the filament, a thin stalk with a single vascular bundle. The androecium is diadelphous, since the posterior stamen is free and other nine stamens are with united filaments from the base to nearly more than half of their length while the anthers are free. The stamens form an open tube enclosing the long ovary. The gynocium is composed of single carpel and the ovary is one locule. Placentation is marginal.

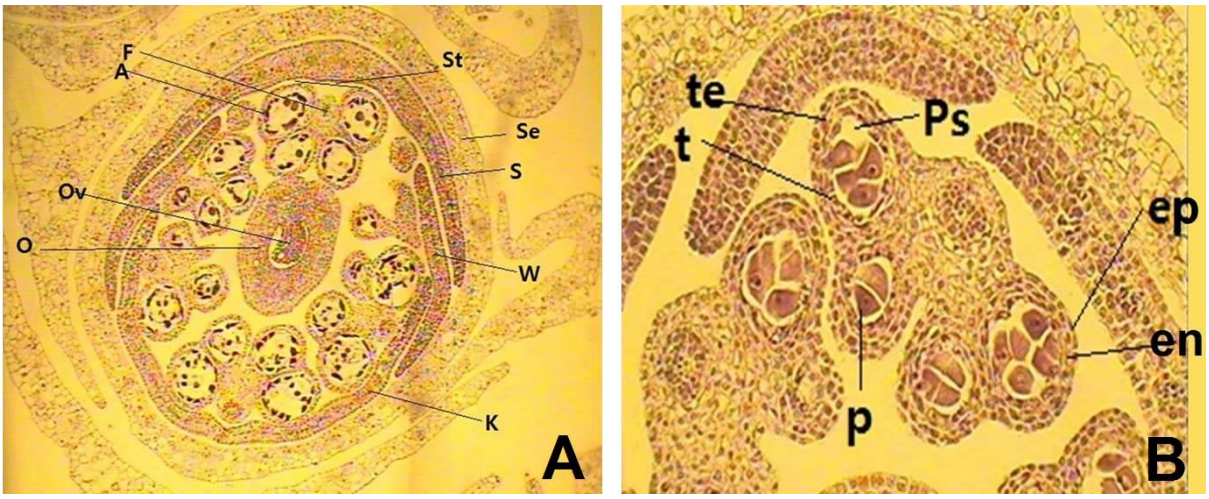

Figure 4. Transverse section (objective X_{10}, X_{40}): **A**, Flower; **B**, Anther. [F: Filament, A: Anther, Ov: Ovary, O: Ovule, St: Secretory tapetum, Se: Sepal, S: Standard, W: Wing, K: Keel, te: Temporary, t: Tapetum, Ps: Pollen sac, ep: Epidermis, en: Endothesium, p: Pollen grain]

Anther anatomy

Microscopic observation showed that anther consists of four compartments or locule, i.e. anthers were tetrasporangiate (Fig. 4A). The young and mature anther wall, which laid under the single-layered epidermis, consisted of three layers: endothecium, temporary layer and tapetum (Fig. 4B). The epidermis was present throughout anther development. However, the temporary layer and tapetum degenerated before or during miosis leaving only the endothecium and the epidermis (Fig. 5A). The endothecium developed fibrous thickening on the radial and inner tangential walls when microspore development was at the uninucelate stage and enabled the mature anthers to dehisce and the pollen to be dispersed. Anther dehiscence started from the broken septum. The broken wall resulted in an opening through which the pollen grains were released.

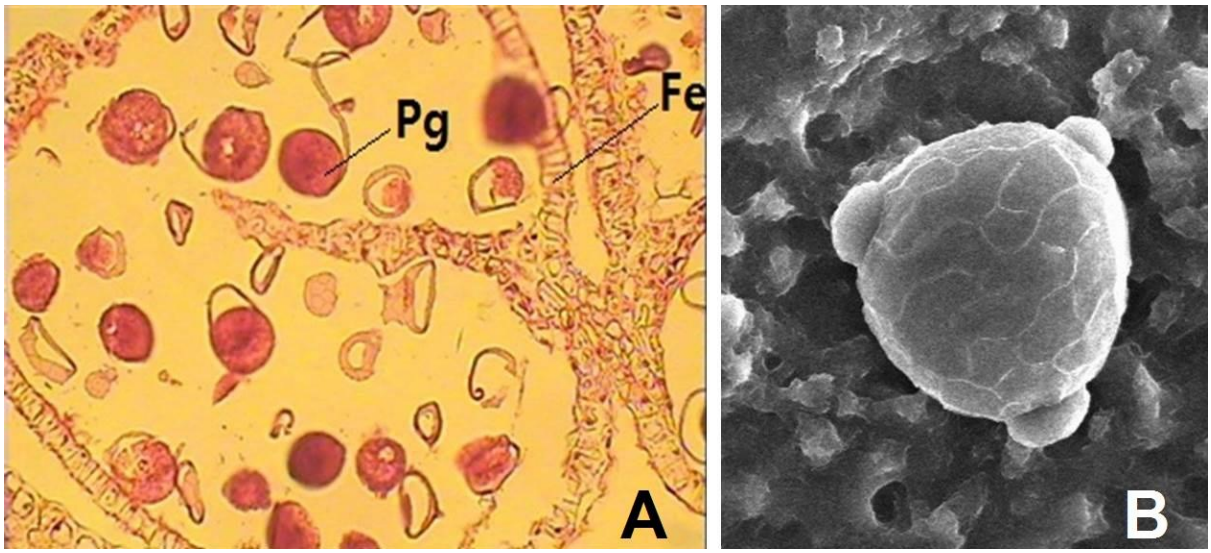

Figure 5. A, Anther dehiscence section (objective X_{40}); **B**, Pollen grain morphology (Zone Mag 1.08KX). [Pg: pollen grain, Fe: Fibrous endothesium]

Pollen grain morphology

Pollen grain is circular shaped, tricolpate, surface has regulated pattern and surface shows reticulum (Fig. 5B).

DISCUSSION AND CONCLUSION

In this study were investigated stem, petiole, leaf and flower anatomy. The studies proved many valuable results. There are many unicellular trichomes in our samples stem and petiole transverse section. Trichomes are hair-like appendages that develop from cells of the aerial epidermis and are produced by most plant species (Werker 2000). Trichomes can serve protection against damage from herbivores (Levin 1973, Traw & Dawson 2002). The morphology and density of leaf trichomes vary considerably among plant species, and may also vary among populations and within individual plants. The structure of trichomes can range from unicellular to multi-cellular, and the trichomes can be straight, spiral, hooked, branched, or un-branched (Southwood 1986, Werker 2000). trichomes may also increase resistance to abiotic stress. They may increase tolerance to drought by reducing absorbance of solar radiation (Ehrlinger 1984, Choinski & Wise 1999, Benz & Martin 2006). In our plant were observed pericyclic fibrous, circular collenchyma cells and intrafascicular cambium. These tissues are in many member *Fabaceae* families (Fahn 1990, Nassar *et al*. 2010). Devadas & Brck (1972) and Ataslar (2004) reported that vascular bundles form a continuous ring. Our results are agreement in this about vascular bundles which are located on one ring. Petiole anatomy characteristic are showed many results. In our plant petiole transverse section is vascular bundles in different parts (adaxial, abaxial and secondary bundles in above of petiole). The number and size of these bundles are different. Shaheen (2006), who reported variations in the number of secondary vascular bundles in the petioles of some Mimosoid species and was used as a distinguishing character among the taxa. There are pericyclic fibre, collenchyma cells and cambium in our plant petiole structure. Öznur *et al.* (2011) examined and compared the petiole of 7 taxa belonging to the Lamiaceae family. They observed some differences in the petiole shape, arrangement and number of vascular, trichome types and the presence of collenchyma. The number of collenchyma layers and position, which is of taxonomic importance (Shaheen 2007). The our sample leaf anatomy is showed that epidermis cells shape is rectangular polygonal whereas in *Lathyrus aphaca* was characterized by epidermal cells with wavy configuration on both adaxial and abaxial surfaces, in *Vicia faba* the epidermal cells on adaxial leaf surface were smooth, longitudinal and linear in shape while cells with wavy outline were found on abaxial surface. *Melilotus indica* could be delimited by highly undulating epidermal cells on adaxial surface and cells with slight undulations abaxially. *Trifolium alexandrianum* stays apart by possessing epidermal cells with polygonal configuration. Idu *et al.* (2000) described the epidermal morphology and the structure and development of stomata in 10 species of Fabaceae. According to them the epidermal cells varied from irregular to straight-walled and in some taxa sinuous patterns were observed. In our sample palisadic layers number is more than spongy cells. The structure and ontogeny of the stomata has been studied in 26 species of Rubiaceae by Bahadur *et al*. (2008) in relation to the irorganographic distribution. The stomata are mostly paracytic on the leaves. In our plant also stomatal type is paracytic and distribution stomata is on both sides of the leaf. This position is also observed in some species of *Acacia* (Shaheen 1995).

Stomatal density is also important. In general, the anatomical features observed on the leaves are consistent with those of Metcalf & Chalk (1950) and Philipson (1963) for the description of leaf anatomy of Leguminosae (Fabaceae). However stomatal diversity is useful at all levels of taxonomic hierarchy. The secretory cells are in stem, petiole and leaf of our examined sample. According to Baran & Ozdemir (2006) secretory cells in the phloem in the structure of leaves, stem and petiole taxonomic information in grouping the different plant taxa.

Flower

The *Vigna radiata* L. (ML2017 genotype) has papilionoied corolla. Our findings are in agreement with Tucker (1987) findings that reported most Fabaceae flowers are papilinoid-type. The anther characteristics of our sample are those typical of a legume flower. In the early stage of anther wall development, the hypodermal cells divided periclinally to form the primary parietal and primary sporogenous cells. The sporogenous cells divided and differentiated into abundant microspore mother cells (microsporocytes) giving rise to a large number of pollen grains (Barth 1990). Meanwhile, the primary parietal cells divided periclinally to form and inner secondary parietal cells underwent further division resulting in the endothecium and on temporary layer. The inner secondary parietal cells developed into the secretory tapetum, the food rich layer of cells (Prakash 1987). In general anatomical charecteristics are very important and this features could be used in diagnostic key of taxa at all taxonomic levels.

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