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JUSTIFICATION OF THE TAXONOMIC ASSIGNMENT OF MUNGBEAN PLANT TO THE GENUS *Vigna*, Savi
III. ANATOMICAL FEATURES.

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ABSTRACT

In this study the anatomical features of mungbean and cowpea plants were used as a taxonomic evidence to justify the assignment of mungbean plant to the genus *Vigna* rather than to the genus *Phaseolus*. Both mungbean and cowpea plants are similar in the following anatomical characters:

The roots have a tetrarch protostele. The stem outline is comprised of six ridges alternating with similar number of furrows. The vascular bundles are arranged in a ring of six large size collateral bundles located opposite to the ridges, in addition to some minor bundles in between. The pith comprises the stem core. The secondary growth takes place in nearly a continuous cylindrical form. Leaves are dorsiventral. Palisade tissue consists of 2-3 layers. Stomata are of paracytic type. The midrib is convex at both sides. Petiole is pentagonal in outline with two lateral wings at the corners of the adaxial side. Flower, fruit, and seed structures are identical.

Phytochemical, morphological and anatomical evidences given in this 3-parts study suggest the acceptance of the scientific name: *Vigna radiata* (L.) Wilczek for mungbean plant.

Keywords: *Vigna*, *Phaseolus*, mungbean, cowpea, anatomy and taxonomy.

INTRODUCTION

Fabaceae are a large and widely distributed family, which is well defined by its floral and fruit characters. A great deal of research on members of the family has been published, including investigations about the taxonomic identity of mungbean plant. Although Verdcourt (1970) recognized the name *Vigna radiata* (L.) Wilczek to mungbean formerly *Phaseolus radiatus* L. and also known as *Phaseolus aureus* Roxb., the taxonomic identity of mungbean has long been disputable and its affinity to *Phaseolus* or *Vigna* is a topic of argument.

Sakr (1971) stated that the taproot of cowpea has a uniseriate epidermis followed by 9-11 layers cortex ending with a well-defined endodermis. The pericycle consists of one layer and the stele is tetrarch. Secondary roots initiate in the pericycle opposite to the protoxylem, their primary and secondary growth structure are similar to those of the tap root, but with lesser amount of tissues. The mature nodule consists of a central bacteriodal tissue, surrounded by a cortical zone in which there are two vascular strands, which connect it with the root stele.

The stem directly below the shoot apex of 2-week-old plants has 6 furrows and 6 ridges. The uniseriate epidermis has stomata and develops glandular hairs. The cortex consists of 2 regions, an outer with thick walled collenchyma layers, and an inner region consists of parenchyma cells. There are 6 major bundles located opposite to the ridges; alternating with 6 minor

ones lying opposite to the furrows. There are groups of weakly developed phloem between the bundles. Several idioblasts lying within or close to the phloem are observed.

The mature leaflet consists of 2 epidermal layers with one-layered palisade tissue, and 4-6 layers of spongy tissue in between. The large bundle of the midrib consists of a rather big bundle opposite to a smaller one. The phloem occupies a wider area than the xylem, which consists of 3-9 rows each with 2-8 vessels. The midrib is reinforced only by collenchyma and no fibrous cells are present. Perianth of the mature pod in transverse section could be divided into exocarp, mesocarp and endocarp.

This is the third paper in a series of investigations about the most acceptable classification of mungbean plant, where anatomical features are introduced as a taxonomic evidence added to other evidences offered in the two previous parts (El-Sahhar *et al*., 2002 a and b).

MATERIALS AND METHODS

Source of used seeds and various fieldwork procedures were previously mentioned (El-Sahhar *et al*., 2002 b).

A full microscopical analysis was carried in this study to evaluate the similarity between the histological structure of mungbean and cowpea plants. Samples representing different plant organs were taken periodically throughout the growing season. The study included the following:

1- The main root, 1 cm. below the hypocotyl.
2- The main stem represented by terminal, median and basal internodes.
3- The mature prophyll and the apical leaflet of the foliage compound leaves number 3, 6 and 9 on the main stem, and secondary branches and the petiole. Type of stomata was defined using epidermal peals.
4- Flower bud.
5- Mature fruit and seed.

Microtechnique procedures given by Nassar and El-Sahhar (1997) were followed. Specimens were killed and fixed for at least 48 hrs. in F.A.A. (10 ml. Formalin, 5 ml. Glacial acetic acid, 85 ml. Ethyl alcohol 70%). After fixation, materials were washed in 50% ethyl alcohol and dehydrated in a normal butyl alcohol series before being embedded in paraffin wax (m.p. 56-58 ^{o}C). Transverse sections, which were cut on a rotary microtome to a thickness of 20µ, were stained with safranin/light green before mounting in Canada balsam. Slides were analyzed microscopically and photomicrographed.

RESULTS AND DISCUSSION

Structure of the main root: The structure of root system of both mungbean and cowpea plants is almost similar. The taproot of 1-week-old seedlings has an uniseriate epidermis of tabular shaped cells. Cuticle on the outer walls and stomata are absent. Some epidermal cells prolong to form the typically unicellular root hairs. The cortex is composed of 7-8 layers of thin–walled irregular parenchyma cells with well–developed intercellular space system.

The innermost layer of the cortex is the endodermis, an uniseriate zone of small barrel–shaped cells forming a distinct layer surrounding the stele. Next to the endodermis lies a layer of thin–walled parenchyma cells forming the pericycle. The vascular bundle is radial. Xylem and phloem occur in separate patches arranged on alternate radii, intervened by small parenchyma cells. The latter forms the conjunctive tissue. The bundle is tetrarch since 4 patches of xylem alternate with an equal number of patches of phloem. Protoxylem vessels occur towards the periphery and metaxylem towards the centre, thus showing centripetal mode of differentiation from the procambium. This is the typical exarch xylem of roots. The central portion is occupied by a metaxylem vessel, so that all the plates of xylem are joined forming a solid core. Hence it is regarded as a protostele.

At the age of 2 weeks (Figure 1), the primary structure is completed. The cortex is characterized by the presence of large air chambers. There is a well-defined layer of endodermis, followed by 1– layered pericycle. The vascular cylinder is more compact and the 4 xylem ridges are joined forming a solid core. Each xylem ridge is comprised of 8 to 10 vessels. Secondary growth takes place in the common way. The cambium originates on the inner side of the phloem forming strands of 2 to 3 layers as a result of tangential divisions of the parenchymatous cells. The cambium is completed as a zigzag band by the inner cells derived from the pericycle layer at places opposite to the xylem poles. The cambial strips located on the inner face of the phloem begin to function, producing a few numbers of xylem vessels towards the inside and small amount of secondary phloem towards the outside.

At the age of 3 weeks, formation of secondary xylem and phloem proceeds. Fibres appear as small groups adjacent to primary phloem. By formation of secondary xylem opposite the phloem the cambium is moved outwards, and eventually its circumference becomes circular in the transverse section. At the same time, the cortex is ruptured at different regions.

When plants are 4 weeks old, the epidermis as well as the cortex are completely sloughed off, and a continuous well-defined periderm arising in the pericycle is present. Secondary thickening proceeds and a cambial zone of about 4 layers is seen. The secondary elements are deposited in considerable quantities and the xylem being more in amount than the phloem. The formation of parenchymtous rays from the cambium, which is originated in the pericycle opposite to the xylem ridges, is clearly obvious in this stage of secondary growth. Noteworthy to mention that tanniniferous cells are present in primary and secondary phloem.

The secondary thickening is more prominent as tested plants were 6 weeks old (Figure 2). In this stage of growth, the root comprises mainly of a vascular cylinder surrounded by a periderm. The vessels of secondary xylem are seen aggregated in radial rows and the ground tissue where the vessels are embedded consists of strands of parenchyma cells intermingled with others of fibres. At this age, the phloem region possessed more fibrous strands and small groups of tanniniferous cells.

fig1

fig2

As far as the authors are aware no detailed study dealing with the anatomical structure of mungbean root was previously carried out. However, the root structure of cowpea given by Sakr (1971) is in harmony with the previously mentioned description.

Structure of the main stem.

The apical internode: The structure of the main stem, 6 weeks old, of both studied plants is almost similar. The internode directly below the shoot apex represents the primary structure of the main stem. The transverse sections shown in Figure (3) reveal that the stem surface directly below the shoot apex, is strongly rigid and fluted. It has ridges alternating with 6 furrows constituting its general outline. The uniseriate epidermis, the outermost zone is consisting of tabular cells attached end on end without leaving intercellular spaces. The epidermal cells are vacuolated and covered with a thin layer of cuticle. Stomata of paracytic type are present at the same level of the epidermis. Trichomes of non-glandular hairs and others of glandular hairs with a head of 3 to 4 cells rich in cytoplasm, and with large nuclei are observed (cowpea). The ridges of the stem mainly consist of collenchyma of nearly 4 layers of cells. The cortex at the furrows between the ridges is composed of 2 layers of collenchyma cells underlying the epidermis followed by 5 to 6 layers of thin–walled parenchyma cells. The pericycle consists of 2 to 3 layers of thin–walled compact parenchyma cells. The vascular bundles are arranged in a ring, being separated from each other by wide panels of parenchyma tissue, which is a part of the ground tissue. There are 6 major collateral bundles located opposite to the ridges. In addition, there are 3 to 4 minor collateral bundles between any of 2 major ones lying opposite to the furrows. The major bundles has 18 to 28 vessels in nearly parallel rows, each comprised of 3 to 5 vessels whereas, the minor bundle has 1 to 3 rows each contains 2 to 4 vessels. The primary phloem of the vascular bundles, especially the major ones, contains an outer portion consisting mainly of different sized parenchyma cells. The sieve tubes with their companion cells are occasionally seen. The inner portion of the phloem, which in nearer to the xylem, comprises mainly of groups of sieve tubes with their companion cells separated by thin walled parenchyma cells. It is worthy to note that tanniniferous cells are observed in the inner portion of the cortex as well as in the primary phloem (mungbean). The pith consists of polygonal parenchyma cells with small triangular intercellular spaces. The pith is connected with the cortex by medullary rays 5 to 6 wide.

The median internode: The main stem at its median portion is often ribbed, but the ribs are comparatively smaller in size than those associated with the apical internode (Figure, 4). The epidermal cells as well as the collenchyma and parenchyma cells of the cortex are keeping pace with the increase in the stem girth. The epidermal cells respond to the increase in stem diameter stresses by tangential enlargement and radial divisions.

fig3

fig4

Also, many of both kinds of cortical cells show elongation in the tangential direction accompanied sometimes by radial divisions. Paracytic stomata are present at the same level of the epidermis. Trichomes of non-glandular hairs are observed (mungbean). They are spine–like in shape. The collenchyma tissue in the ridges and furrows becomes well defined and the innermost layer of the cortex, the starch sheath, is easily recognized. The pericyclic cells show their transformation into fibres in the ridges and furrows. Thus, an incomplete ring of fibrous strands is developed.

The stele consists of 27 to 30 collateral bundles arranged in a ring, being separated from one another by the ground tissue. The bundles are relatively different in size. The large bundle has 28 to 31 vessels, while the intermediate bundle has 15 to 18 vessels and the small one has 4 to 6 vessels. The vessels of each bundle are arranged in parallel rows. A complete cambial ring is formed by the continuity of the interfascicular cambium with the fascicular one.

The pith consists of large polygonal parenchyma cells with relatively small intercellular spaces.

The basal internode: Secondary growth is shown in the basal internode of the main stem as continuous cylindrical form (Figure, 5). Therefore, the stem at its basal portion looses its distinct outline; *i.e*., disappearance of the ridges and furrows. The epidermis, which still having intact cells shows active dilation accompanied by radial division to accommodate with the increase in stem circumference. Trichomes are not observed. The cortex consists of 6 to 7 layers of thin–walled parenchyma cells. The pericyclic fibres formed discontinuous ring. The secondary phloem increases considerably in amount and it appears in pyramidal–like forms separated by ray parenchyma whose outer cells are conspicuously dilated.

The secondary xylem has an increased amount of vessels present in nearly radial rows, and the ground tissue where the vessels are embedded is formed of lignified parenchyma cells intermingled with small groups of fibres. Xylem rays are composed of 2 to 3 layers of well-lignified parenchyma cells. The primary xylem is recognized abutting on the pith. Tanniniferous cells are often situated in the secondary phloem. The pith consists of polygonal parenchyma cells, which tend to decrease in size towards the periphery. Small triangular intercellular spaces are visible.

At the extent of the author's knowledge no detailed studies dealing with the anatomical structure of mungbean stem are available. However, the description of the stem structure of cowpea given by Sakr (1971) is in accordance with the present findings.

Structure of the leaf.

Leaf blade: Transverse sections of mature prophyll as well as of apical leaflet of the foliage compound leaf number 3, 6 and 9 on the main stem and the lateral branches of mungbean and cowpea were examined. It was obvious that all these leaves generally have the same structure. They are composed of 3 tissue systems.

fig5

i- Epidermal tissue system consists of the epidermal layers occurring on the adaxial (upper) and abaxial (lower) sides.

ii- The ground tissue system, which is known as the mesophyll tissue, is always differentiated into columnar palisade parenchyma on the adaxial side and irregular or isodiametric spongy parenchyma on the abaxial side and this means that the leaves are dorsiventral.

iii- The vascular tissue system is composed of vascular bundles, which are usually collateral, and form the skeleton of the leaf on which other tissues, the ground tissues, remain inserted. At the midrib the principle vascular vein develops. In addition, smaller lateral ones constitute the reticulate system of venation.

Transverse sections through the median portion of the mature prophyll, 2 weeks old, are shown in Figure (6). It is clear that both the upper and lower epidermis are uniseriate, composed of nearly compactly arranged rectangular cells with thin recorded cuticularised outer walls. Stomata occur on both sides, being more frequently present on the lower epidermis. Trichomes of non-glandular hairs are present on both surfaces. They are of unicellular type. The mesophyll is differentiated into palisade and spongy cells.

The palisade tissue consists of 3 to 4 layers of cells elongate perpendicularly to the surface of the blade being characterized by an abundance of chloroplasts. The palisade tissue occupies 2/3 of the whole thickness of the mesophyll. The spongy tissue occurs towards the lower epidermis and consists of 2 to 4 layers of chlorenchymatous cells with wide intercellular spaces.

At the midrib region, both upper and lower epidermis are convex. The bundle in the midrib is the largest in the prophyll and the lateral ones decrease in size towards the margins. The vascular bundle is oriented with the xylem directed toward the adaxial surface and the phloem toward the abaxial one. Phloem elements are more in the larger bundles than in the smaller ones, and the xylem includes 4–5 rows of vessels in the formers and from 2–3 in the latters. The vascular bundle of the midvein is embedded in a ground tissue of parenchyma cells with a mass of collenchyma cells underlying the two epidermis. Whereas, the smaller bundles are directly embedded in the mesophyll.

Figure (7) illustrates the anatomical structure of leaflet blade of the studied plants. There are 2 epidermal layers on the adaxial surfaces of the leaflet. Each is uniseriate, composed of a row of compactly–set tabular cells. The outer walls are cutinised and possess thin cuticle. Stomata occur on both surfaces, being more numerous on the lower epidermis than on the upper one. They are of paracytic type. Esau (1959) also mentioned this type of stomata. At the midrib region, both upper and lower epidermis are convex. Trichomes are present. They are of uniseriate non-glandular hairs. The palisade tissue consists of 3 layers of slender cells of dense plastids, occupying 2/3 of the whole thickness of the mesophyll. It is clear that the palisade tissue is partly extended in the midrib region. The rod – shaped crystals recorded by Metcalfe and Chalk (1959) to be present in the palisade tissue of *Vigna* leaves were not observed.

fig6

fig7

The spongy tissue is composed of 2–4 layers of chlorenchymatous loosely arranged cells with many wide intercellular spaces. There is a mass of collenchymatous cells below the adaxial and abaxial epidermis at the midrib region. Therefore, the included bundle, the principle one, is not directly embedded in the mesophyll as do the smaller ones. The midrib bundle consists of a larger strand opposite to a smaller one. The xylem consists of about 3 to 7 parallel rows depending on the size of the strand, each with 3 to 5 vessels. The phloem occupies a wider area than the xylem due to the presence of a relatively larger amount of big-sized parenchyma cells in proportion to sieve tubes and companion cells.

Leaf petiole: The petiole of mungbean and cowpea is almost pentagonal in outline with 2 wing-like appendages at the adaxial corners (Figure, 8). It is bounded by an uniseriate epidermis of nearly square-shaped cells. The outer walls of the epidermis are somewhat thickened and covered with a thin layer of cuticle. Stomata and trichomes are present. Trichomes are similar to those found on the main stem and the leaf blades (mungbean). The ground tissue consists mostly of relatively large parenchyma cells. The angles, beneath the epidermis, consist mainly of collenchyma. Generally, there are from 2 to 3 layers of collenchyma cells underlying the epidermis and the number being more at the corners. Inside the collenchyma there is a zone of 2 to 3 layers of collenchyma cells.

The vascular tissues are formed of 5 main collateral bundles at the corners. The 2 bundles located at the adaxial side are laterally enlarged in size and arc-like in shape. The other 3 main bundles are located at the abaxial side each opposite to a corner. A fibrous cap is obviously abutting on the phloem of each of these 3 bundles. It is clear that xylem vessels are arranged in radial rows each consists of 3 to 5 vessels. Worthy to mention that 2 accessory small bundles develop in the ground tissue of the 2 wing-like appendages at the adaxial side.

Anatomical structures of leaflet blade and leaf petiole of cowpea given by Sakr (1971) are in harmony with those of the present study.

Structure of the floral bud: The floral bud of mungbean and cowpea is surrounded by 3 free bracts (Figure, 9). Each bract is bounded by an uniseriate epidermis and has 7 collateral bundles embedded in the ground tissue at nearly equal distance from each other. The bundle in the midrib of the bract is the largest and the lateral ones (3 at the left side and the same number at the right side) decrease in size towards the margins. The calyx consists of 5 united sepals comprised of 2 epidermal layers and 3–4 layers of ground tissue in between. There are numerous traces which extending through the ground tissue. The corolla is papilionaceous with 1 posterior petal (the standard), 2 lateral petals (the wings) and 2 lower united anterior petals (the keel). Each segment consists of two epidermal layers of nearly square-shaped cells surrounding 2–4 layers of slightly elongated parenchymatous cells forming the mesophyll. Many traces are extending through the mesophyll.

The stamens are 10; each consists of 2-lobed tetrasporangiate anther borne on the filament, a thin stalk with a single vascular bundle. The androecium is diadelphous, sine the posterior stamen is free and the other 9 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 upper parts of the filaments bend toward the banner.

The gynoecium is composed of a single carpel and the ovary is of 1 locule. Placentation is marginal. The ovary wall consists of an outer epidermis with rather palisade like cells with dense cytoplasm. Next to the epidermis are several layers of compact vacuolated cells followed by a few layers of compact and small non-vacuolated cells. There is an innermost layer abutting on the inner epidermis. It has square or upright weakly vacuolated and compact cells with conspicuous nuclei. The inner epidermis has square cells, with dense cytoplasm and clear nuclei. The ovary contains 2 bundles at the placenta side (the dorsal bundles) and a single one at the opposite side (the ventral bundle). The ovule has 1 integument, which consists of an outer epidermis, an inner one, and a parenchyma tissue lying in between. The integument surrounds the nucleus with its embryo sac.

Structure of the fruit and seed: Transverse sections of the mature fruit (pod) of mungbean and cowpea are shown in Figure (10). The exocarp includes the epidermis and a subepidermal layer of 1–2 cells in thickness; both are composed of thick-walled cells. The mesocarp consists of several layers lying next to the exocarp. The component parenchymatous cells of these layers are characterized by their rather thick and slightly lignified walls. They are tangentially elongated and deeply stained. The mesocarp contains the vascular traces supplying the pod. The endocarp comprises the remainder of the parenchymatous cells of the fruit wall, the pericarp, and the inner epidermis. The parenchymatous cells are weakly stained being thin-walled and much enlarged in different planes. The inner epidermis though still intact, its constituent cells become weakly outlined and thus could be hardly described.

The seed coat, the integument of mature ovule, differentiates into variety of distinct layers. The outermost layer, the epidermis, remains uniseriate and develops into the palisade layer characteristic of leguminous seeds. It is composed of macrosclereids with unevenly thickened walls. The cells of the subepidermal layer differentiate into the so-called columnar cells (pillar or hourglass cells). The layers beneath the subepidermal layer are of bigger and tangentially elongated parenchyma cells with the innermost layers being largely pressed. The vascular system is well developed; it is an extension of the vascular bundle from the funiculus to the chlazal region where it branches. Finally, the inner epidermis with very poorly outlined cells with rather thick lignified walls. It is worthy to note that, two palisade layers occur in the hilum region. The outer of these is derived from the funiculus and the inner belongs to the seed coat. Moreover, a compact group of cells of unknown role occurs in the hilum region. They are referred to by Esau (1959) to be tracheids.

The seed coat envelops the embryo, which consists of 2 large cotyledons, plumule and the radicle. The cotyledons have big thin-walled cells, rich in starch grains of conspicuously large size.

The above description of the seed coat is in general agreement with that given by Hector (1936) as well as by Esau (1959) and Sakr (1971).

CONCLUSION

The present study indicates that the anatomical structure of mungbean and cowpea is almost similar. Both plants shared the following characters:

The root has a tetrarch protostele. The stem outline is comprised of six ridges alternating with similar number of furrows. The vascular bundles are arranged in a ring of six large size collateral bundles located opposite to the ridges, in addition to some bundles in between. The pith comprises the stem core. The secondary growth takes place in nearly a continuous cylindrical form. Leaves are dorsiventral. Palisade tissue consists of 2–3 layers. Stomata are of paracytic type. The midrib is convex at both sides. Petiole is pentagonal in outline with two lateral wings at the corners of the adaxial side. Flower, fruit and seed structures are identical.

In essence, electrophoretic analysis of seed storage protein given in the first part of this series (El-Sahhar *et al*., 2002 a) proved that mungbean is more related to cowpea with similarity of 21% compared with 9% between mungbean and kidney bean. These findings suggest that the assignment of mungbean to the genus *Vigna* is quite reasonable. Further chemical and morphological studies (El-Sahhar *et al*., 2002 a and b) and anatomical features added more evidences toward the assignment of mungbean to the genus *Vigna* rather than to the genus *Phaseolus*. Hence, the scientific name *Vigna radiata* (L.) Wilczek is the most acceptable and recognized as the name of choice for mungbean.

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تقنين الوضع التصنيفي لنبات فول المانج وتبعيته الى جنس اللوبيا *Vigna* Savi

ثالثاً: الخصائص التشريحية

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استخدمت الخصائص التشريحية في هذه الدراسة كدليل تصنيفي نحو تقنين تبعية نبات فول المانج الي جنس اللوبيا *Vigna* وليس الي جنس الفاصوليا *Phaseolus* . تماثل كل من نبات فول المانج ونبات اللوبيا في الخصائص التشريحية التالية:

الجذر رباعي أذرع الخشب – الحدود الخارجية للساق ذات ستة بروزات تتبادل مع ذات العدد من الانخفاضات – ترتب الحزم الوعائية في حلقة من ست حزم جانبية كبيرة الحجم مقابل البروزات بالاضافة الى بعض الحزم صغيرة الحجم فيما بينها - يشغل النخاع مركز الساق – يحدث النمو الثانوي على شكل أسطوانة متصلة في غالبيتها – الاوراق ظهرية بطنية – يتركب النسيج العمادى من ٢-٣ طبقات – الثغور من الطراز المتوازى – العرق الوسطى محدب الجانبين – عنق الورقة خماسي الشكل ذو جناحين لدى أركان الجهة الداخلية (العليا) للورقة – يتماثل تركيب الزهرة والثمرة والبذور.

ترجح الدلائل المورفولوجية والتشريحية والكيميائية النباتية في هذه الدراسة ذات الثلاثه أجزاء قبول الاسم العلمى: *Vigna radiata* (L.) Wilszek لنبات فول المانج.