

Morphological and Anatomical characteristics of Leaves of *Glycine max* (L.) Merr.

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Abstract

The morphological and anatomical characteristics of leaves of *Glycine max*(L.) Merr., which was a member of the family Fabaceae (Subfamily-Papilionoideae) were studied. The specimens were collected from Yezin, Naypyitaw. The plant was an annual erect herb. Leaves were pinnately trifoliolate compound and stipules acute. The flowers were bisexual, zygomorphic, pentamerous, hypogynous and papilionaceous. Pods are oblong, dehiscent. The vascular bundle of petiole was a collateral type, accompanied by two accessory bundles present in adaxial two lateral prominent wings. In surface view of lamina, paracytic type of stomata was observed from both surfaces. The vascular tissue system of midribs was composed of two opposite bundles, with their xylem group abutting on one another. The bundle was arc-shaped, collateral type. The morphological and anatomical characteristics of leaves of the *Glycine max* (L.) Merr. were useful to provide the identification and classification of this plant.

Key words: *Glycine max*, Morphological, Anatomical

INTRODUCTION

The family Papilionaceae is the largest of the three families of the order Leguminales. This includes about 375 genera. The members of the family are distributed in the temperate region or both northern and southern hemispheres. Usually the herbs belonging to this family appear and flower in winter season (Pandey, 2000).

Glycine max (L.) Merr. is cultivated throughout China and temperate and tropical regions. This specie provides 35 % for the human protein. It is also an important source of oil and was used extensively and industrially (Zhengyi & Raven 2013). *Glycine max* (L.) Merr. is used a very good food. The bean has high oil content and the kernel is rich in proteins. Among the useful products may be included soya-sports, soya-milk and soyabean oil from the seeds, extensively used in paints, varnish and enamel industries; refined oil was used as a salad or cooking oil

(Pandey, 2012).

The stomata of *Glycine max* (L.) Merr. are predominantly paracytic. Mesophyll is dorsiventral with biseriate palisade parenchyma and extended cells in many sizes, disposed perpendicularly to the limbo surface. The spongy parenchyma has 3 or 4 layers of irregular cells, with prevalent intercellular spaces (Polizel *et al.* 2011 and Stolf *et al.* 2016). Leaves have paracytic stomata on both surfaces. Mesophyll consists of 2 – 3 layers of elongated palisade cells and 3 – 6 layers of isodiametric spongy parenchymatous cells with large intercellular cavities (Makbul *et al.*, 2011).

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Median veins containing two opposite systems of bundles, with their xylem groups abutting on one another. In petiole, accessory bundles are observed from the wings of the species of *Glycine* (Metcalfe & Chalk 1950). Midrib is semi-circular and has 1-layered epidermis. Arc-shaped vascular bundle is surrounded by orbicular and thin-walled parenchymatous cells. Phloem has some differentiated cells with thickened walls (Makbul *et al.* 2011). The midrib of *Glycine max* (L.) Merr. is presented as xylem in the central portion with external phloem, confirming the organization of the vascular system of dicotyledonous and a thin layer of sclerenchyma under the phloem (Stolf *et al.*, 2016).

The aim and objectives of this research are to study the morphological and anatomical characteristics of leaves of *G. max* (L.) Merr. and to provide the specific information of morphological and anatomical characteristics for the identification of this plant.

MATERIALS AND METHODS

The species of *Glycine max* (L.) Merr. were collected from Yezin, Naypyitaw in November 2017. After the collection, the specimens were studied and identified by using literatures such as Dassanayake (1991), Qi-ming & Nian-he (2008) and Zhengyin & Raven (2013).

After the collection, some of the specimens were studied in fresh stage and others were pressed and dried at room temperature as well as preserved in 50% ethyl alcohol for further study. After killing and fixation, the small portions of petioles, lamina and midribs were thoroughly washed in running water for overnight and dehydrated through a series of tertiary butyl alcohol solutions. Then, they were infiltrated in a series of paraffin wax of which melting points were 49°C, 55°C and 60°C respectively. They were kept in oven for overnight or 24 hours. The specimens were then embedded in the 60°C paraffin wax and cut into 15-25 µm thick sections by using a rotary microtome. These thin sections were double-stained with the combination of safranin and fast green solutions. After staining, the sections were permanently mounted under a cover slip with a Canada Balsam in xylol and kept dried for a few weeks on slide tray. The dehydration, infiltration, embedding, staining and mounting were made according to Johansen's method (1940).

The parts of specimens were macerated by warming in equal volume of 50% hydrogen peroxide solution and 50% glacial acetic acid solution according to Franklyn's method (modified) (Berlyn & Miksche 1976). Photomicrographs were prepared from microtome sections and macerated materials.

RESULTS

Morphological characteristics of *Glycine max* (L.) Merr. (Figure 1)

Myanmar Name	-	Pe bok
English Name	-	Soy bean
Flowering period	-	November and December

Annual erect herb, 30.0 – 120.0 cm high; stems and branches are densely brown hirsute. Leaves are pinnately trifoliolate compound, alternate; stipules acute, 0.4 – 0.7 cm by 0.2 – 0.4 cm, basifixed, persistent, pubescent; petioles 3.7 – 20.0 cm long, pubescent; stipels setaceous, 0.2 – 0.4 cm long, pubescent; leaflets elliptic – lanceolate, cuneate or rounded at the base, entire along the margin, acuminate at the apex, sparsely hirsute on both surfaces;

terminal leaflets 6.0 – 17.8 cm by 3.9 – 8.7 cm; lateral leaflets 6.8 – 12.7 cm by 4.8 – 8.7 cm. Inflorescence is axillary raceme; peduncles terete, about 0.3 cm long, pubescent. Flowers are bisexual, zygomorphic, cyclic, pentamerous, hypogynous, bluish to purple; pedicels about 0.1 cm, pubescent; bracts lanceolate, 0.4 – 0.7 cm long; bracteoles linear – lanceolate, 0.2 – 0.5 cm long. Calyx is campanulate, 5-lobed; tube about 0.2 cm long, pubescent; lobes acute, about 0.1 cm long, pubescent. Corolla is papilionaceous; standard abovate, about 0.4 cm by about 0.4 cm, apex emarginated, bluish, glabrous; wings obovate – oblong, about 0.3 cm by 0.2 cm, distinct auricle, white, glabrous; keels oblong, about 0.3 cm long, purple, glabrous. It has stamens 10 (9+1), diadelphous, inserted; free filament filiform, about 0.3 cm long, white, glabrous; anther ditheous, basifixed, white, dehiscent longitudinally. It has carpel 1; ovary superior,

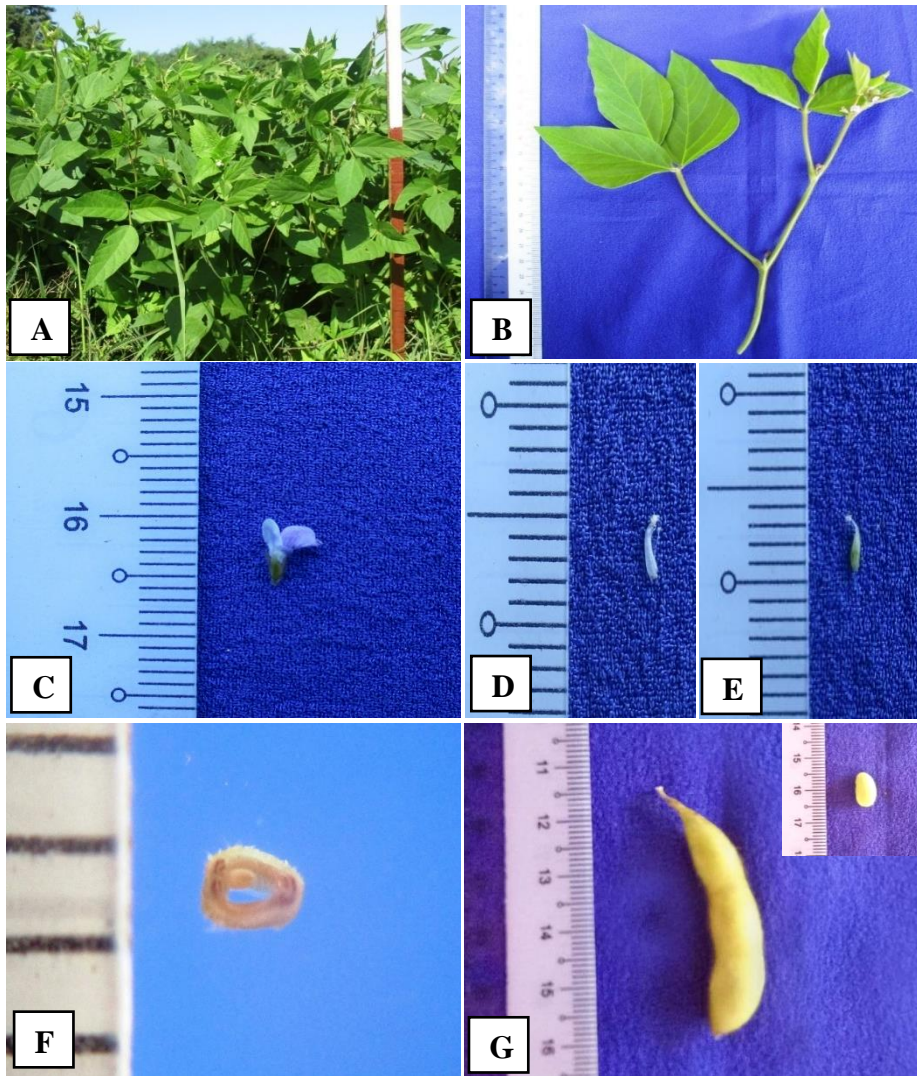


Figure 1. Morphological characteristics of *Glycine max* (L.) Merr.

- A.** Habit **B.** Inflorescence **C.** L.S of Flower
- D.** Androecium **E.** Gynoecium **F.** T.S of ovary
- G.** Pod and seed

oblong, about 0.3cm long, unilocular, many ovules in the locule on the marginal, placentae, hairy; style linear, about 0.1 cm long; stigma hairy, yellow. Pods are oblong, 4.0 – 8.0 cm long, 3 or 4 seeded, dehiscent, slightly curved, and densely yellow-brown hirsute. Seeds are

elliptic-ovate, 0.3 – 0.5 cm long, green when they are young, pale brown when mature, glabrous.

Anatomical characteristics of petioles of *Glycine max* (L.) Merr.

In transverse section, the petiole is oval shaped in outline with prominent wings at the adaxial side, 2250.0 – 3125.0 μm in length, 2500.0 – 3125.0 μm in width. It can be distinguished into dermal, ground and vascular tissue systems as shown in Figure 2.

Dermal Tissue System: It is composed of epidermal cells and trichomes. In transverse section, it has epidermis 1-layered on surfaces, the cell barrel or rectangular in shape, compact, 7.5 – 25.0 μm in length, 10.0 – 33.75 μm in width, outer and inner wall convex, anticlinal walls straight. Trichomes are non-glandular, unicellular and uniseriate, 187.5 – 500.0 μm in length, 50.0 – 62.5 μm in width.

Ground Tissue System: It is composed of outer collenchymatous and inner parenchymatous tissues. Collenchymatous tissue is below the epidermis, 2-4-layered, the layers are 33.75 – 75.00 μm thick, angular type, the cells polygonal or oval in shape, 10.00 – 27.50 μm in length, 10.00 – 31.25 μm in width; the inner parenchymatous cells are below the collenchymatous layers, 2 to 5-layered, the layers are 56.25 – 137.5 μm thick, the cells oval or rounded in shape, 18.75 – 41.25 μm in length, 18.75 – 47.5 μm in width and intercellular spaces are also present.

Vascular Tissue System: Vascular bundle embeds in the ground tissue in which bundles arranged in a ring. It consists of 20 – 22 bundles, 5 large bundles opposite the ridges and 15 – 17 small bundles, collateral type, accompanied by 2 accessory bundles present in adaxial 2 lateral prominent wings. Each bundle is oval in shape, 300.0 – 600.0 μm in length, 275.0 – 600.0 μm in width; phloem lies outside and xylem inside. Phloem is composed of 3 to 11-layered; the layers are 18.75 – 93.75 μm thick, the cells polygonal in shape, 5.0 – 10.0 μm in radial diameter, and 3.75 – 10.25 μm in tangential diameter. Phloem has sieve tubes, companion cells, and phloem parenchyma and phloem fibres. Xylem is composed of 3 to 9-layered; the layers are 33.75 – 100.0 μm thick, the cells polygonal in shape, and 22.5 – 58.75 μm in radial diameter, 18.75 – 47.50 μm in tangential diameter. Xylem is composed of vessel elements, tracheids, xylem fibres and xylem parenchymas. Vessel elements have thick walls, lateral walls straight, end walls oblique or transverse, thickening spiral or scalariform, perforation plate's simple, 110.0 – 650.0 μm (mean 296.3 μm) in length,

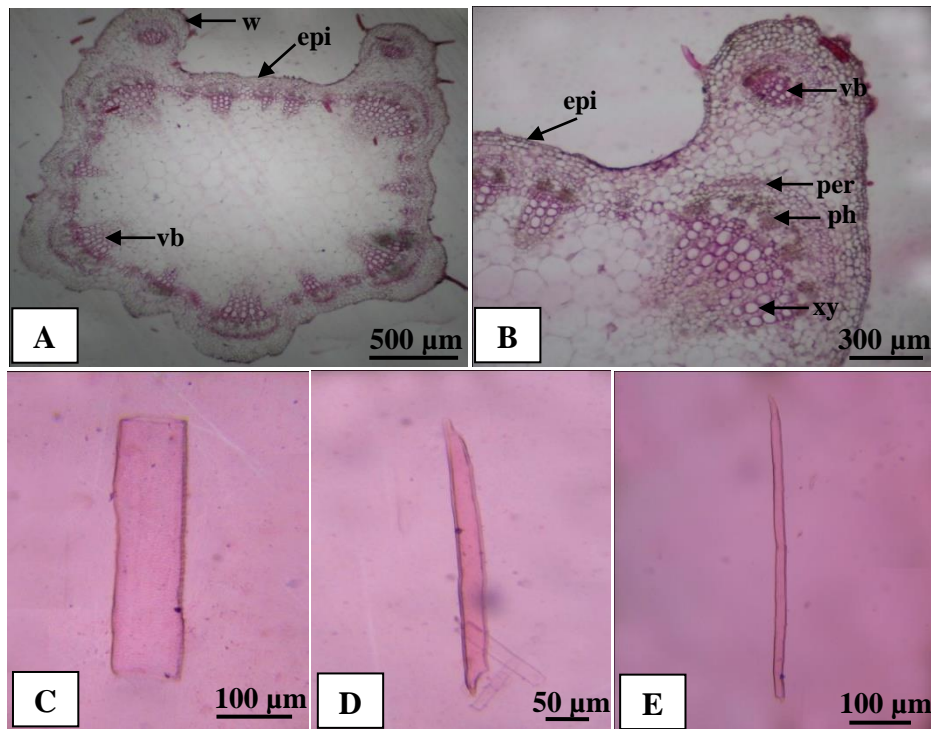


Figure 2. Internal structures of petiole of *Glycine max* (L.) Merr.

A. T.S of petiole **B.** Close up view of vascular bundles and wing **C.** Vessel element **D.** Tracheid **E.** Fibre
(epi = epidermal cell, per = pericycle, ph = phloem, vb = vascular)

20.0 – 90.0 µm (mean 49.1 µm) in width. Tracheids are elongate, lateral wall long, lateral walls straight, end walls acute, thickening spiral or pitted, 150.0 – 1150.0 µm (mean 374.4 µm) in length, 10.0 – 35.0 µm (mean 20.0 µm) in width. Fibres are long, lateral walls straight, end walls acute, 330.0 – 2200.0 µm (mean 731.6 µm) in length, 10.0 – 20.0 µm (mean 14.3 µm) in width.

Anatomical characteristics of lamina of *Glycine max* (L.) Merr.

In transverse section, the lamina is dorsiventral with reticulate venation, 120.0 – 200.0 µm thick. It can be distinguished into dermal, ground and vascular tissue systems as shown in Figure 3.

Dermal Tissue System: It is composed of epidermal cells, guard cells of stomata, subsidiary cells and trichomes. In surface view, adaxial epidermal cells are parenchymatous, irregular or polygonal in shape, 13.75 – 43.75 µm in length, 10.0 – 52.50 µm in width; cell walls slightly wavy, cells compact, intercellular spaces are also present. Abaxial epidermal cells are parenchymatous, irregular or polygonal in shape, 13.75 – 57.50 µm in length, 12.5 – 68.75 µm in width and cell walls are more waiver than adaxial surfaces, cells compact, intercellular spaces present; stomata paracytic type on both surfaces, more abundant on abaxial surface; guard cells on adaxial surface 18.75 – 28.75 µm in length, 5.00 – 8.75 µm in width. Guard cells are on abaxial surface, 16.25 – 25.00 µm in length, 3.75 – 7.50 µm in width. Subsidiary cells are on adaxial surface, 22.50 – 56.25 µm in length, 3.75 – 18.75 µm in width; subsidiary cells are on abaxial surface, 27.50 – 66.25 µm in length, 3.75 – 21.25 µm in width.

Trichomes are present on both surfaces, unicellular, 150.0 – 550.0 μm in length, 10.0 – 20.0 μm in width. In transverse section, both upper and lower epidermis have got 1-layered; adaxial epidermal cells are barrel shaped, 8.75 – 17.5 μm in length, 10.0 – 47.5 μm in width, anticlinal walls straight, outer and inner walls convex. Abaxial epidermal cells are barrel shaped, 8.75 – 22.5 μm in length, 12.5 – 46.25 μm in width, anticlinal walls straight, outer and inner walls convex and cuticle is thin on both surfaces.

Ground Tissue System: Mesophyll is differentiated into palisade and spongy parenchyma. Palisade parenchyma has 2 or 3 layers, the layers are 31.25 – 68.75 μm thick; the cells are elongate, 12.5 – 37.5 μm in length, 5.0 – 11.25 μm in width, intercellular spaces are also present. Spongy parenchyma has 3 to 6 layers, the layers are 40.0 – 75.0 μm thick, and the cells are rounded or oval in shape, 6.25 – 15.0 μm in length, 6.25 – 16.25 μm in width, chloroplast abundant, intercellular spaces present.

Vascular Tissue System: Vascular bundles of lateral veins are embedded in the mesophyll tissues. They are collateral type and 50.0 – 60.0 μm in length, 40.0 – 50.0 μm in width. Phloem is composed of sieve tubes, companion cells, and phloem parenchyma and phloem fibres. Xylem is composed of vessel elements, tracheids, and xylem parenchyma and xylem fibres. Vessel elements has thick walls, lateral walls straight, end walls oblique or transverse, thickening spiral or scalariform, perforation plates simple, 75.0 – 500.0 μm (mean 251.0 μm) in length, 15.0 – 45.0 μm (mean 24.1 μm) in width. Tracheids are elongate, lateral walls straight, end walls bluntly acute, thickenings spiral or pitted, 135.0 – 700.0 μm (mean 379.8 μm) in length, 10.0 – 25.0 μm (mean 16.6 μm) in width. Fibres are long, lateral walls straight, end walls acute, 200.0 – 1800.0 μm (mean 676.2 μm) in length, 10.0 – 25.0 μm (mean 13.3 μm) in width.

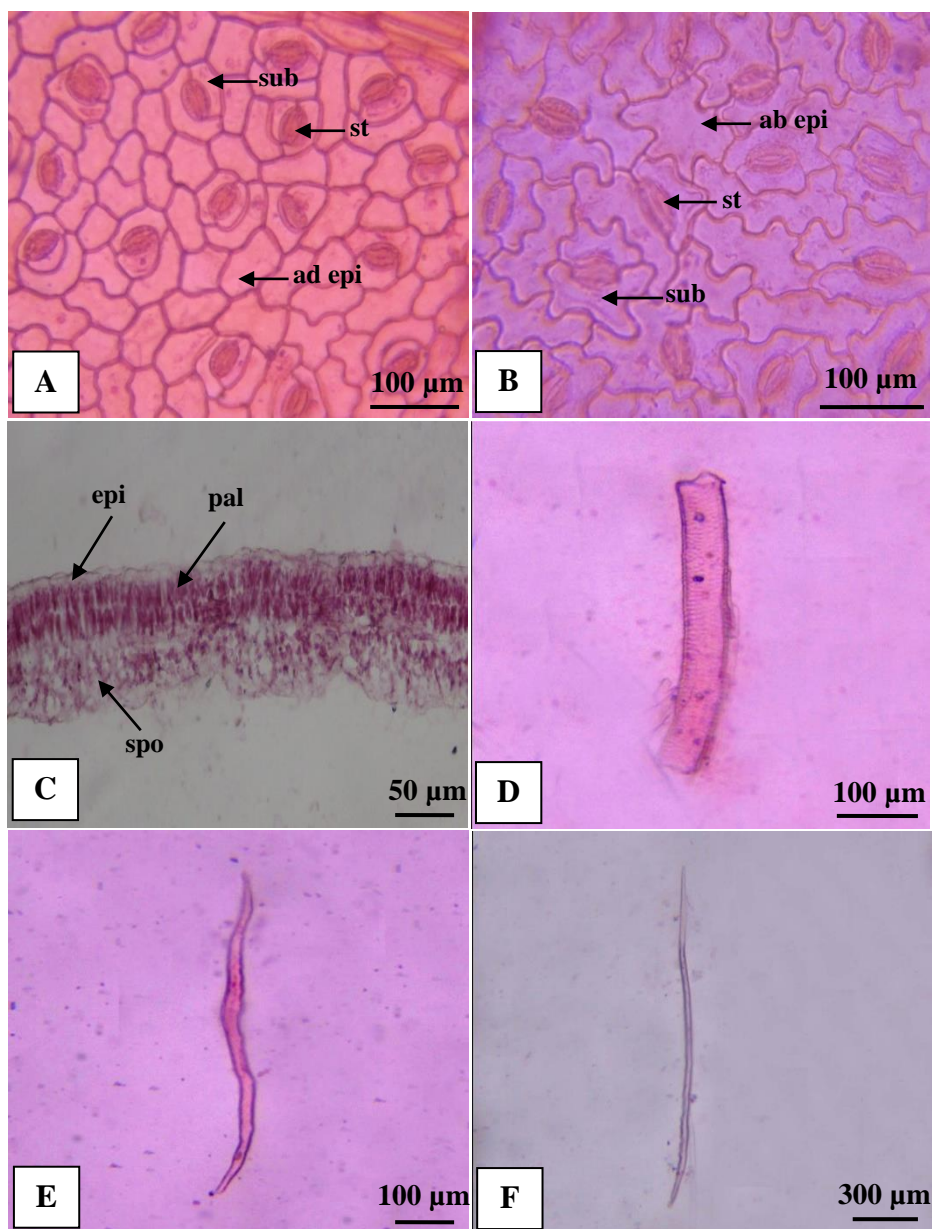


Figure 2. Internal structures of lamina of *Glycine max* (L.) Merr.

A. Adaxial surface **B.** Abaxial surface **C.** T. S of lamina
D. Vessel element **E.** Tracheid **F.** Fibre

(ab epi = abaxial epidermal cell, ad epi = adxial epidermal cell,
 epi = epidermal cell, pal = palisade parenchyma cell, spo = spongy
 parenchyma cell, st = stoma, sub = subsidiary cell)

Anatomical characteristics of midribs of *Glycine max* (L.) Merr.

In transverse section, the midrib is semicircular-shaped in outline with circular at the abaxial side and prominently protrude at the adaxial side, 850.0 – 1125.0 µm in length, 500.0 – 812.5µm in width. It can be distinguished into dermal, ground and vascular tissue systems as shown in Figure 4.

Dermal Tissue System: It is composed of epidermal cells. In transverse section, both upper and lower epidermis have 1-layered, the cells barrel shaped, 5.00 – 28.75 μm in length, 8.75 – 31.25 μm in width, outer and inner walls are convex and anticlinal walls are straight.

Ground Tissue System: It is composed of outer collenchymatous and inner parenchymatous tissues. Collenchymatous cells are below the adaxial epidermis, 1 to 3-layered, the layers are 16.25 – 72.50 μm thick; the cells are oval or rounded in shape, 15.0 – 40.0 μm in length, 15.0 – 26.25 μm in width. Collenchymatous cells are above the abaxial epidermis, 1 to 4-layered, the layers are 22.5 – 93.75 μm thick; the cells are oval or rounded in shape, 17.5 – 40.0 μm in length, 17.5 – 31.25 μm in width. Inner parenchymatous cells are above the vascular bundle, 4 to 14-layered, the layers are 200.0 – 350.0 μm thick; the cells are oval or rounded in shape, 16.25 – 47.5 μm in length, 15.0 – 47.5 μm in width. Parenchymatous cells are below the vascular bundle, 3 to 5-layered, the layers are 100.0 – 162.5 μm thick; the cells are oval or rounded in shape, 18.75 – 65.00 μm in length, 27.5 – 50.0 μm in width.

Vascular Tissue System: Vascular tissue system is composed of two opposite bundles, with their xylem groups abutting on one another; one large bundle situated above the abaxial side, arc-shaped, collateral type, 350.0 – 500.0 μm in radial diameter, 325.0 – 500.0 μm in tangential diameter, phloem lying outside and xylem lying inside; phloem has 4 to 10-layered, the layers are 37.5 – 75.0 μm thick, the cells are polygonal, 3.75 – 10.00 μm in length, 2.5 – 12.5 μm in width, phloem is composed of sieve tubes, companion cells, phloem parenchyma and phloem fibres; xylem has 3 to 6-layered, the layers are 75.00 – 175.00 μm thick, the cells are polygonal in shape, 12.5 – 41.25 μm in length, 12.5 – 35.0 μm in width, xylem is composed of vessel elements, tracheids, xylem parenchyma and xylem fibres. Vessel elements has got thick walls, lateral walls straight, end walls oblique or transverse, thickening spiral or scalariform, perforation

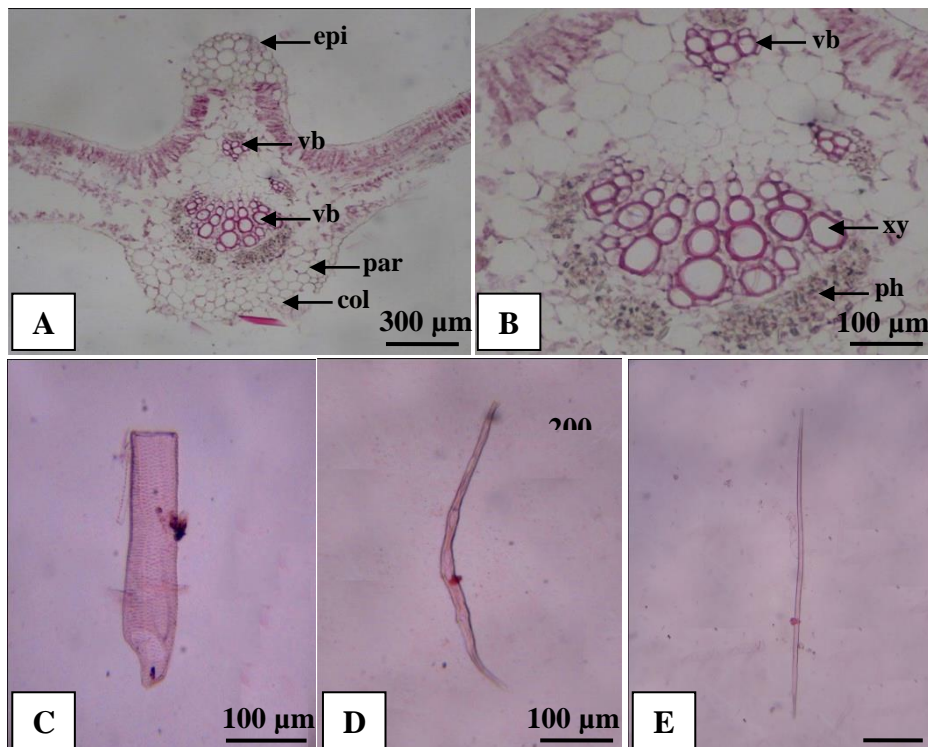


Figure 4. Internal structure of midrib of *Glycine max* (L.) Merr.

A. T. S of midrib **B.** Close up view vascular bundle

C. Vessel element **D.** Tracheid **E.** Fibre

(col = collenchyma cell, epi = epidermal cell, par = parenchyma cell, ph= phloem, vb = vascular bundle, xy = xylem)

plates simple, 100.0 – 450.0 μm (mean 212.4 μm) in length, 15.0 – 75.0 μm (mean 36.5 μm) in width. Tracheids are elongate, lateral walls straight, end walls bluntly acute, thickening spiral or pitted, 110.0 – 750.0 μm (mean 277.2 μm) in length, 10.0 – 45.0 μm (mean 20.0 μm) in width. Fibres are long, lateral walls straight, end walls acute, 300.0 – 2550.0 μm (mean 880.3 μm) in length, 10.0 – 25.0 μm (mean 13.6 μm) in width.

DISCUSSION AND CONCLUSIONS

The morphological and anatomical characteristics of leaves of *Glycine max* (L.) Merr. were studied in this research. *Glycine max* (L.) Merr. belongs to the family Fabaceae (Subfamily-Papilionoideae). The specimens were collected from Yezin, Naypyitaw during flowering and fruiting period of this plant in November 2017.

The habit of *G. max* (L.) Merr. was annual erect herb. The leaves were pinnately trifoliolate compound. The shape of stipules was acute. The inflorescence was axillary raceme. The colour of flowers ranges from bluish to purple. The flowers were bisexual, zygomorphic, pentamerous and hypogynous. The calyx of the species were campanulate, 5-lobed and corolla papilionaceous. The stamens were diadelphous. The anther was dithecous and basifixed. The ovaries were superior and many ovules in the locule on the marginal placentae. Pods were oblong, dehiscent, 3 or 4 seeded and densely yellow brown hirsute. These characters were agreed with those mentioned by Dassanayake (1991), Qi-ming & Nian-he (2008) and Zhengyi & Raven (2013).

The anatomical characteristics of petioles, laminae and midribs were composed of dermal, ground and vascular tissue systems. In transverse sections of petioles, the epidermis has 1-layered and barrel or rectangular shaped cells. In the ground tissue system, cortex was composed of outer collenchymatous and inner parenchymatous tissues. The transverse sections of petioles were observed from the wings at the adaxial side, the bundles arrange in a ring, collateral type, accompanied by 2 accessory bundles present in adaxial 2 lateral prominent wings. These characters were agreed with Metcalfe & Chalk (1950).

In transverse section of lamina, the epidermis was 1-layered with barrel shaped cells. In surface view of laminae, stomata were observed as paracytic types on both surfaces and more abundant on abaxial surfaces. Mesophyll consists of 2 or 3-layered of elongated palisade cells and 3 to 6-layered of spongy parenchymatous cells. These characters were agreed with Polizel *et al.* (2011), Makbul *et al.* (2011) and Stolf *et al.* (2016).

The midribs were semicircular-shaped in outline. The vascular tissue system of midribs has two opposite bundles, with their xylem groups abutting on one another. The bundle was arc-shaped, collateral type. The phloem lie outside and xylem inside. These characters were agreed with Metcalfe & Chalk (1950), Makbul *et al.* (2011) and Stolf *et al.* (2016).

In this research, the outstanding morphological and anatomical characteristics of leaves were found to be useful to help in identification and classification of flowering plants. Furthermore, it is hoped that the finding of this research will support the requirement for the information of *G. max* (L.) Merr.

Acknowledgements

We are grateful to Dr Theingi Shwe (Rector, Hinthada University), Dr Yee Yee Than (Pro-Rector, Hinthada University) and Dr Cho Kyi Than (Pro-Rector, Hinthada University), for her invaluable advice and kind suggestions. We would also like to express our gratitude to Dr Thidar Oo, Professor and Head, Department of Botany, Hinthada University, for her permission, invaluable advice and providing all the departmental facilities. We are also thankful to Dr Wah Wah Myint, Professor, Department of Botany, Hinthada University, for her suggestions.

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