

Macro- and micromorphological study of *Salvia hispanica* L. cultivated in Egypt

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Running Title: Botanical study of *Salvia hispanica* L.

Abstract

The macro- and micro-morphological characters of leaf, petiole, stem, calyx, corolla, androecium, gynoecium, seed and root of *Salvia hispanica* L. are presented with their diagnostic elements by which the plant can be easily identified in both entire and powdered form. The fascinating thing during the current research is the determination of the presence of different types of non-glandular and glandular trichomes in different organs, also the epidermal cells of leaves and seeds contain mucilage content, the stem contains pericyclic fibers and the root is covered by metaderm layer.

Keywords: *Salvia hispanica*, leaf, stem, inflorescence, seed, root

Introduction

Family Lamiaceae (Labiatae, Mint) constitutes about 236 genera and 6900 to 7200 species worldwide (Venkateshappa and Sreenath, 2013). Many of these species have important uses in medicine, culinary and cosmetics (Abdelkader et al., 2014).

The genus *Salvia* is one of the largest genera in family Lamiaceae, has about 1000 species (El-Sahhar et al., 2016), it has antibacterial, antiseptic, astringent, cytotoxic, anti-inflammatory, antiviral, cardiovascular, sedative tranquilizing, anticonvulsant, spasmolytic, carminative, anti-oxidant and anti-diabetic effects (Hamidpour et al., 2014 ; Topcu et al., 2008).

Salvia hispanica L. is an annual herbaceous plant commonly known as (Chia) that is native of southern Mexico and northern Guatemala (Ayerza and Coates, 2005). Chia seeds have been recommended for use because of its content of fatty acids, proteins, dietary fiber and antioxidants and consider as an excellent sources of Omega-3 FA and mucilage (Silva et al., 2016 ; Coorey et al., 2012).

Concerning the botanical study of *S. hispanica* L., only few short notes were reported in the text books about its macro-morphology, from this view we decided to carry out the study of this plant. Therefore, the aim of our work was studying the macro- and micro-morphological study of *S. hispanica* L. leaves, petiole, stem, inflorescence, seed and root aiming to find out

the diagnostic elements by which the plant and these organs can be easily identified in both entire and powdered forms.

MATERIALS AND METHODS

Plant material.

The fresh leaves, stems, inflorescences, seeds and roots of *Salvia hispanica* L. were collected from Mushtohor farm, Tokh, Egypt march 2018. The plant was identified and verified by Dr. Hussein Abdelbaset, (Professor of plant taxonomy, Faculty of Science, Zagazig University). A voucher specimen (Lam.S-10) was deposited in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University, Egypt. Samples of different organs were well-preserved in a mixture of ethanol/ glycerin (1:1, v/v) and stored in a firmly sealed container for botanical study.

Reagents

5% potassium hydroxide solution. Glycerine, Phloroglucinol / Conc.HCl, saffranin and 66% Sulphuric acid.

RESULTS AND DISCUSSION

I. Macro-morphology

The leaves (Fig. 1, B&C) are lime green, simple, opposite decussate, exstipulate, having semicircular with two appendages petiole. The petiole measures 5 to 7 cm in length, their blade is lanceolate-ovate measures 4 to 9 cm in length and 2 to 6 cm in width. They have acuminate apex, serrated margin, reticulate venation and symmetric base. The lower surface is lighter in

color, more hairy with a prominent midrib than the upper surface. They have aromatic odor and slightly bitter taste.

The stem (Fig. 1D) is erect, hairy attaining around 120 to 170 cm in height. The diameter of stem is 0.5 to 0.7 cm at the base and 0.2 to 0.3 cm near the apex. It is square and branched bearing both inflorescence and foliage branches.

The inflorescence (Fig. 1E) is a raceme that generates at the axils of upper leaves on the main stem and lateral branches, forms a whorl flowers at each node, this type is known as verticillaster, it measures around 10 cm in length, 1.5 to 2 cm in width and contains 6-20 zygomorphic flowers.

Bracts are hairy, ovate-acuminate in shape, measure 0.4 to 0.8 cm in length and 0.2 to 0.5 cm in width.

The flowers are hermaphrodite, zygomorphic, and hypogenous with bluish-violet color. The calyx (Fig.1, F&G) is bell shaped with 5 toothed green sepals, they measure 0.5 to 0.7 in length and 0.2 to 0.3 in width, the upper lip is tridentate (Fig.1F) but the lower lip is bidentate (Fig. 1G). The corolla (Fig.1, H&J) is bilabiate tubular in shape, the upper lip is straight (Fig. 1J), short with 2 petals measuring 0.4 to 0.5 cm in length, 0.2 to 0.3 cm in width and length but the lower lip is broad (Fig. 1k), longer than upper lip with 3 petals measuring 0.5 to 0.7 cm in length and 0.3 to 0.4 cm in width. The androecium (Fig.1, J&K) consists of 2 short epipetalous stamens. It has brown anthers measuring around 0.1 cm in length, 0.05 to 0.1 cm in width and yellow filament measuring 0.1 to 0.2 cm in length with long connectives measuring around 0.3 cm in length. The Gynoecium (Fig.1, J, K&L) is yellow in color, consists of bifid stigma, long style, superior bi-carpellary, tetralocular with single ovule in each locule and basal placentation, it measures 0.8 to 1 cm in length. The seed (Fig. 1M) is subspherical, albuminous, consists of seed coat, embryo and endosperm, the hilum is subcircular and crateriform, the seeds measure around 0.2 to 0.3 cm in diameter, the color of seeds is ranging from white to black. The root (Fig. 1N) is perennial tap root with rough surface, dark brown in color and breaks with fibrous fracture. It bears few short tapering adventitious roots.

II. Micro-morphology

1- The leaf

A transverse section of the leaf (Fig. 2A) shows a dorsiventral structure with 1-2 rows of palisade cells beneath the upper epidermis and is interrupted by 2-3 rows of collenchymatous cells in the midrib region. The midrib is more prominent on the lower surface and formed of parenchymatous cells with peripheral collenchymatous cells and transversed longitudinally by crescent-shaped vascular bundle.

The Epidermis:

The epidermal cells of both upper and lower surfaces (Fig. 2, C&D) are rectangular with thin slightly wavy anticlinal wall being wavier in the lower one and are covered with thin smooth cuticle. The neural epidermal cells (Fig. 2, E&F) are polygonal and axially elongated with thin straight anticlinal walls. The cell dimensions are shown in Table (1).

Table (1): dimensions of epidermal cells of *S. hispanica* L. leaf (in micron)

Epidermis	Length	Width	Height
Upper lamina	38- 55	16- 29	6- 25
Lower lamina	20- 41	14- 25	5- 10
Upper neural	30- 59	17- 34	8- 24
Lower neural	30- 63	18- 29	11- 26

Stomata:

The stomata (Fig. 2I) are numerous on both surfaces being more on the lower surface than the upper one. They are diacytic type. The measure of osteole is 8 to 10 μ in length and 3 to 4 μ in width, subsidiary cells measure 40 to 60 μ in length and 7 to 39 μ in width.

Trichomes:

Trichomes (Fig. 2, J&K) are present on both upper and lower epidermises. The non-glandular trichomes are conical, slightly curved, uniseriate, mostly 3 celled and measure 90 to 172 μ in length. The glandular trichomes each with 1-2 celled stalk and 1 to 8 radiating celled globular head (labiate hair) and unicellular or multicellular uniseriate stalk with unicellular head (capitate glandular trichomes). They measure 53 to 109 μ in length.

The Mesophyll:

The mesophyll (Fig. 2A) is dorsiventral. The upper palisade is formed of 1-2 rows of palisade cells. They measure 26 to 40 μ in

length and 5 to 13 μ in width as well as a spongy tissue formed of 1-3 rows of thin walled scattered parenchymatous cells of irregular shape containing chloroplasts, they measure 9 to 20 μ in diameter.

The Midrib:

The Cortical Tissue:

The cortical tissue (Fig. 2A) is formed of parenchymatous cells surrounded by 2-3 rows of collenchyma below the upper epidermis and 1-2 rows of collenchyma above the lower epidermis. Few starch granules are found in the cortex, lenticular in shape and the smaller ones are globular in shape with central hilum and faint striation, aggregated as simple, semi compound and few compound granules (Fig. 2L). **The endodermis** is undifferentiated.

The Vascular Tissue:

The vascular tissue (Fig. 2A) consists of an upper radiated xylem with a phloem band underneath. The xylem (Fig. 2M) is formed of lignified spiral, annular and scalariform vessels measuring 8 to 25 μ in diameter separated by polygonal cellulosic wood parenchyma.

2- The petiole

A transverse section of the petiole (Fig. 2B) is a boat shape in outline with obtuse margins. The petiole has concave upper surface with two wings at its corners while the lower surface is convex with concave region in the middle. It shows an outer epidermis surrounds a cortex with inner parenchyma and outer collenchyma, the main vascular bundle is arc-shaped in the middle of petiole and two small vascular bundles present in the two wings.

The epidermis:

The epidermal cells of both upper and lower surfaces (Fig. 2, G&H) are polygonal with straight anticlinal wall and covered with moderately thick smooth cuticle in upper surface and striated cuticle in lower one. The cell dimensions are shown in Table (2)

Trichomes:

Trichomes (Fig. 2B) are present on both upper and lower epidermises. The types of trichomes are the same types on leaf.

The cortical tissue:

The cortical tissue (Fig. 2B) is formed of parenchymatous cells which are surrounded by 2-3 rows of angular collenchyma and 5-7 rows at the wings.

The vascular tissue:

Each vascular bundle is formed of lignified xylem and cellulosic phloem. The xylem is composed of spiral vessels measuring 10 to 30 μ in diameter that are separated by uni-seriate to bi-seriate medullary rays.

3- The stem

A transverse section of the stem (Fig. 3A) is quadrangular with rounded corners. It shows an epidermis surrounds the cortex which consists of collenchyma in the corners, aerenchyma and parenchymatous tissue. The vascular tissue is composed of prominent vascular bundles in the corners and vascular cambial zone between the corners. The outermost layer of vascular tissue is composed of small lignified pericyclic fibers arranged in clumps followed by narrow band of active phloem then wide band of xylem and pith which is formed of parenchymatous cells and central cavity.

The epidermis:

The epidermis of the stem (Fig. 3B) consists of axially elongated rectangular cells with straight anticlinal wall and covered with thin smooth cuticle. They measure 36 to 77 μ in length, 23 to 25 μ in width and 10 to 19 μ in height.

There are glandular and non-glandular **trichomes** but **stomata** are absent on epidermis.

Trichomes:

The non-glandular trichomes (Fig. 3C) are conical, slightly curved, uniseriate, multicellular mostly 3 celled trichomes. The glandular trichomes (Fig. 3D) each with 1-2 celled stalk and 1 to 8 radiating celled globular head (labiate hair) and unicellular stalk with unicellular head (capitate gland).

The cortex:

The cortex (Fig. 3A) is formed of 5-7 rows of annular collenchyma in the corners, followed by 2-3 rows of aerenchyma. Inwards of aerenchyma there is a very narrow region of parenchymatous cells. The endodermis is undifferentiated.

Table (2): dimensions of epidermal cells of *S. hispanica* L. petiole (in micron)

Epidermis	Length	Width	Height
Upper	43- 77	13- 37	12- 19
Lower	35- 88	11- 23	14- 24

The pericycle:

The pericycle (Fig. 3A) consists of small lignified fibers (Fig. 3E) arranged in clumps. They are spindle-shaped fibers with moderately thick lignified walls, narrow lumen and acute apices. They measure 400 to 430 μ in length and 18 to 40 μ in width.

The vascular tissue:

The vascular bundles (Fig. 3A) are arranged in crescent-shape in the corners, the size of the vascular bundles at the corners is larger than that of the bundles between the corners.

The phloem:

There is a narrow band of active phloem (Fig. 3A) composed of sieve tubes and companion cells.

The cambial zone:

The vascular cambial zone (Fig. 3A) presents inward the cortex at the faces. There are small vascular bundles embedded in vascular cambial zone that connect the corner vascular bundles.

The xylem:

The xylem (Fig. 3A) consists of lignified wood parenchyma in which xylem vessels are scattered with few trachieds. The xylem vessels (Fig. 3F) are lignified, Spiral and reticulate arranged radially in groups measuring 10 to 51 μ in diameter. The wood parenchyma (Fig. 3A) formed of rectangular cells with lignified wall measuring 11 to 24 μ in diameter.

The medullary rays:

The medullary rays (Fig. 3A) are mostly multi-seriate and formed of radially elongated cells which are lignified in the xylem region.

The pith:

The pith (Fig. 3A) is wide and formed of large rounded, thin-walled cellulosic parenchyma measuring 55 to 210 μ in diameter. The center of pith is hollow, this cavity is surrounded by cellular debris indicated that breaking from apoptosis.

Table (3): dimensions of epidermal cells of *S. hispanica* L. calyx and corolla (in micron)

Epidermis	Inner epidermis			Outer epidermis		
	Length	width	Height	Length	width	Height
Calyx epidermis	10- 27	10- 12	4- 6	23- 26	11- 16	11- 21
Corolla epidermis	8- 20	5- 11	5- 14	14- 21	7- 14	22- 25

4- The inflorescence

1-The Calyx

A transverse section of the calyx (Fig. 4A) showed a convex structure. The mesophyll is formed of more or less rounded parenchymatous cells with wide intercellular spaces in sepal tubes and there are small vascular bundles situated in the center of mesophyll.

The epidermis of the calyx:

The outer and inner surfaces (Fig. 4, B&C) are rectangular cells with wavy anticlinal wall and covered with moderately thick smooth cuticle surround 3 to 6 rows of parenchymatous cells.

There are glandular and non-glandular **trichomes** but **stomata** are absent on both epidermises.

The cell dimensions are shown in Table (3)

Trichomes:

Trichomes (Fig. 4, D, E&F) present on the outer surface, they are non-glandular, straight, uniseriate, multicellular mostly 4 celled trichomes (Fig. 4D) measuring 50 to 318 μ in length, non-glandular, curved, unicellular trichomes (Fig. 4E) measuring 75 to 116 μ in length. Glandular trichomes (Fig. 4F) each with 1-2 celled stalk and 1 to 8 radiating celled globular head (labiate hair) and unicellular stalk with unicellular head (capitate gland).

2- The Corolla

A transverse section of the corolla (Fig. 4G) showed that the outer surface is more prominent than the inner one enclosing in between a narrow mesophyll of parenchymatous cells with vascular strands (Fig. 4G) at the center which are formed of lignified spiral vessels and phloem.

The epidermis of the corolla:

The outer and inner surfaces (Fig. 4, H&I) are rectangular cells with straight anticlinal wall and covered with moderately thick smooth cuticle. The cell dimensions are shown in Table (3).

Trichomes:

Trichomes are distributed on the outer surface, non-glandular, straight, uniseriate, multicellular with 2 to 5 cells (Fig. 4J) and glandular trichomes with 1-3 celled stalk and 1 -8 radiating celled globular head trichomes (Fig. 4K).

Table (4): dimensions of epidermal cells of *S. hispanica* L. filament, stigma and style (in micron)

Epidermis	Length	width	Height
Filament epidermis	53- 118	12- 18	5- 14
Stigma epidermis	50- 67	6- 16	6- 9
Style epidermis	56- 93	16- 26	7- 14

3- The Androecium

The filament:

The filament (Fig. 5A) consists of epidermis surrounding a parenchymatous ground tissue which is traversed longitudinally by a vascular strand.

The epidermal cells (Fig. 5B) are polygonal axially elongated cells with straight anticlinal wall and covered with thick smooth cuticle. The cell dimensions are shown in Table (4)

The anther:

The anther (Fig. 5C) consists of two anther lobes; each lobe encloses two pollen sacs and separated by a connective. The anther wall (Fig. 5C) is formed of an exothecium followed by 1-2 rows of endothecium, fibrous layer of anther and a collapsed tapetum. The pollen grains (Fig. 5D) are spherical in shape, smooth, with 2 to 3 germ pores and germinal furrows, yellow in color. They measure 28 μ in diameter.

4-The Gynoecium

The Stigma:

Microscopical examination of the stigma showed bifid stigma that formed of an epidermis enclosing parenchymatous cells. The epidermal cells (Fig. 5E) are elongated polygonal cells, with straight anticlinal walls and covered with thick striated cuticle with papillae in some cells. The cell dimensions are shown in Table (4)

The style:

A transverse section of the style (Fig. 5G) showed a closed style with central specialized secretory tissue and the transmitting tissue, that links the stigma with the centre of the ovary,

and serve as a nutrient-rich tract for pollen-tube growth (Tilton and Horner Jr, 1980). This transmitting tissue is surrounded by two vascular strands. The epidermal cells of the style (Fig. 5F) are elongated rectangular thin walled cells with straight anticlinal wall and covered with thin smooth cuticle. The cell dimensions are shown in Table (4).

The ovary

A transverse section of the ovary (Fig. 5H) is bi-carpellary, tetralocular with 4 ovules, one ovule per locule.

5- The seed

A transverse section of the seed (Fig. 6A) is a rhomboidal to triangular in outline. The seed testa is formed of three layers with mucilage concentrated in the epidermal layer. The epidermal cells (Fig. 6B) are polygonal with wavy anticlinal wall and covered with moderately thick smooth cuticle, these cells measure 16 to 21 μ in length, 9 to 12 μ in width and 2 to 6 μ in height. The second layer of the testa Consists of thick, lignified, sinuous walled sclerenchymatous cells (Fig. 6C). The inner most layer of the testa is formed of thin rectangular cells.

The embryo is axial, regular, with faces of the cotyledons and subdivided into five regions (Fig. 6A). The endosperm is consisting of thick walled round cells, thin walls with protein and lipid bodies. They measure 9.5 to 30 μ in diameter.

6- The root

A transverse section of the root (Fig. 6D) is almost circular in outline. It is formed of an outer brown metaderm, followed by narrow band of cortex, surrounding a cylinder of vascular tissue comprises a narrow outer phloem and a wide inner xylem with cambium in-between.

The metaderm:

The metaderm layer (Fig. 6E) consists of polygonal cells, with no regular arrangement. The cells have thick suberized brownish wall. The cells measure 41 to 77 μ in length, 22 to 44 μ in width and 8 to 22 μ in height.

The cortex:

The cortex (Fig. 6D) is formed of multilayers of rectangular elongated parenchymatic cells, flattened cells with 6-20 rows and they measure 8 to 91 μ in diameter. There are

sclerenchymatic groups (Fig. 6, F&H) distributed in the cortex.

The Vascular Tissue:

The phloem: (Fig. 6D) is considered as narrow band consists of polygonal, thin-walled cellulosic elements.

The cambium between xylem and phloem consists of 1-2 layers of thin-walled tangentially elongated rectangular meristematic cells.

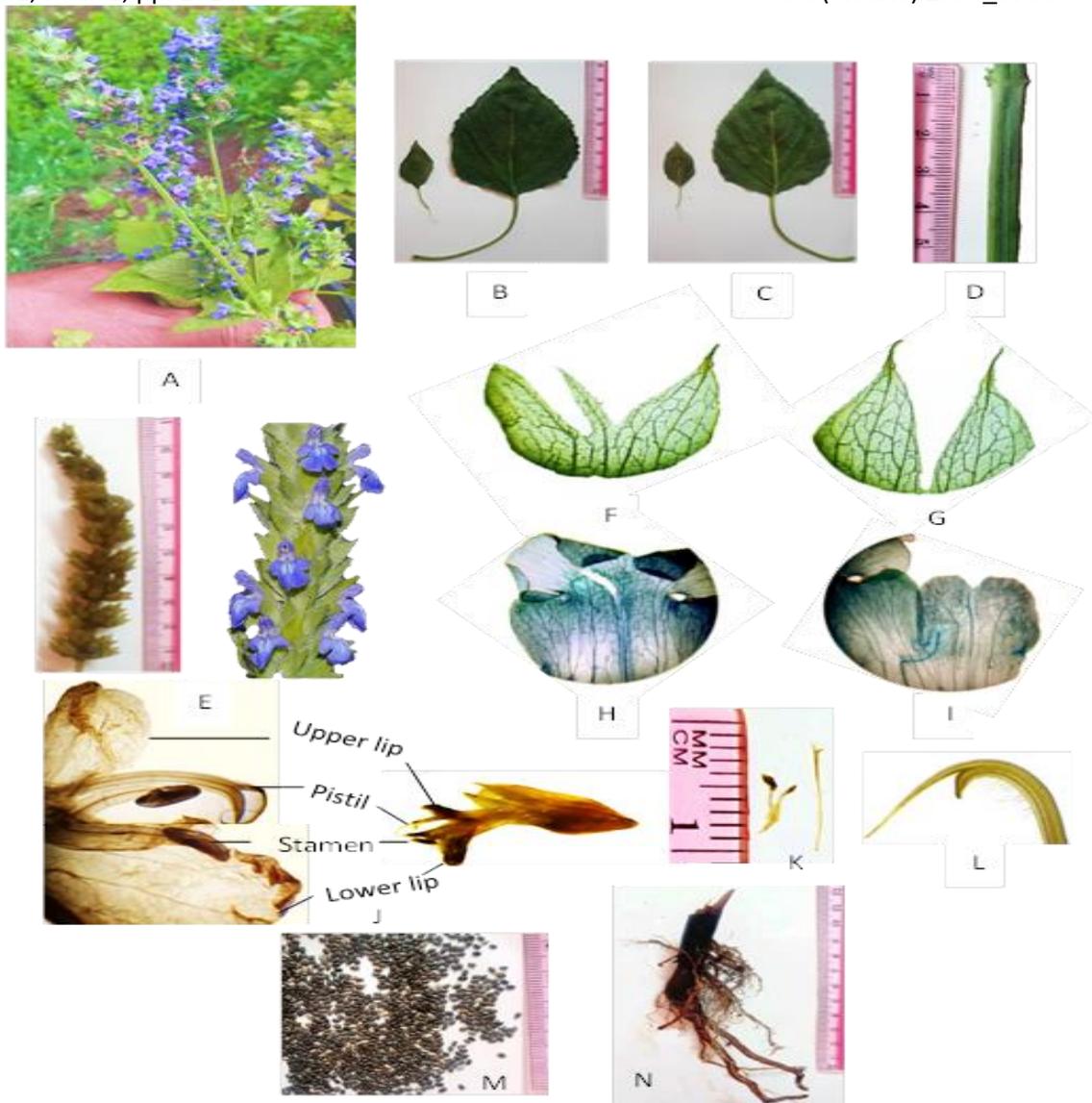
The xylem (Fig. 6D) is wide, comprises the most area of root transverse section, mainly consists of xylem vessels, trachieds and wood parenchyma, transversed radially by pith rays. Xylem vessels (Fig. 6G) are reticulate, diffused and with radial rows. Xylem trachieds (Fig. 6H) are present showing blunt apices and moderately thick lignified walls showing rounded bordered pits. Root anatomy of the family lamiaceae is characterized by presence of pith rays (**Metcalf and Chalk, 1972**). The pith rays (Fig. 6D) are composed of 3-4 cells in width of rectangular cells measuring 7 to 21 μ in diameter.

CONCLUSION

Anatomical study of *Salvia hispanica* L. is characterized by:

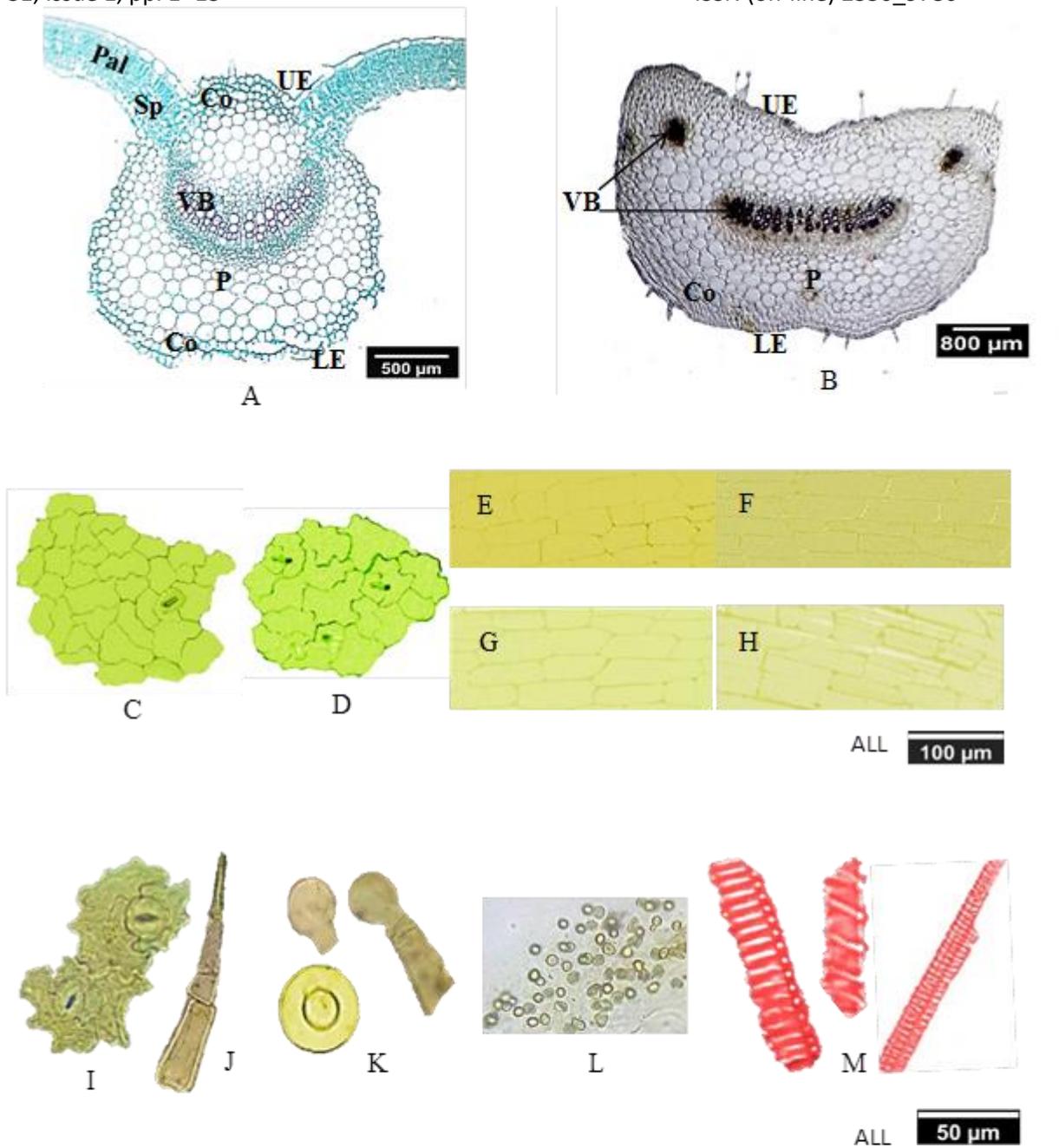
- 1- The leaf shows dorsiventral structure characterized microscopically by fragments of epidermal cells which have anticlinal walls and covered with thin smooth cuticle.
- 2- The stem is hollow, quadrangular characterized microscopically by collenchymatous cells at the corners and presence of pericyclic fibers.
- 3- The florets have two lips in which the lower lip is longer than the upper lip, contain 2 stamens and 1 pistil which is characterized by bifid stigma, long style and bi-carpellary ovary.
- 4- The seed is rich with mucilage in epidermal layer.
- 5- The root is characterized by presence of metaderm, narrow cortex and wide xylem region.

There are many types of trichomes, non-glandular trichomes with unicellular to multicellular trichomes and glandular trichomes in different shapes.



A, Photograph for aerial parts; B, upper surface leaf; C, lower surface leaf; D, stem; E, inflorescence; F, upper lip sepals ($\times 50\mu\text{m}$); G, lower lip sepals ($\times 50\mu\text{m}$); H, upper lip petals ($\times 50\mu\text{m}$); I, lower lip petals; J, floret ($\times 50\mu\text{m}$); K, reproductive organs; L, pistil ($\times 40\mu\text{m}$); M, seeds; N, root.

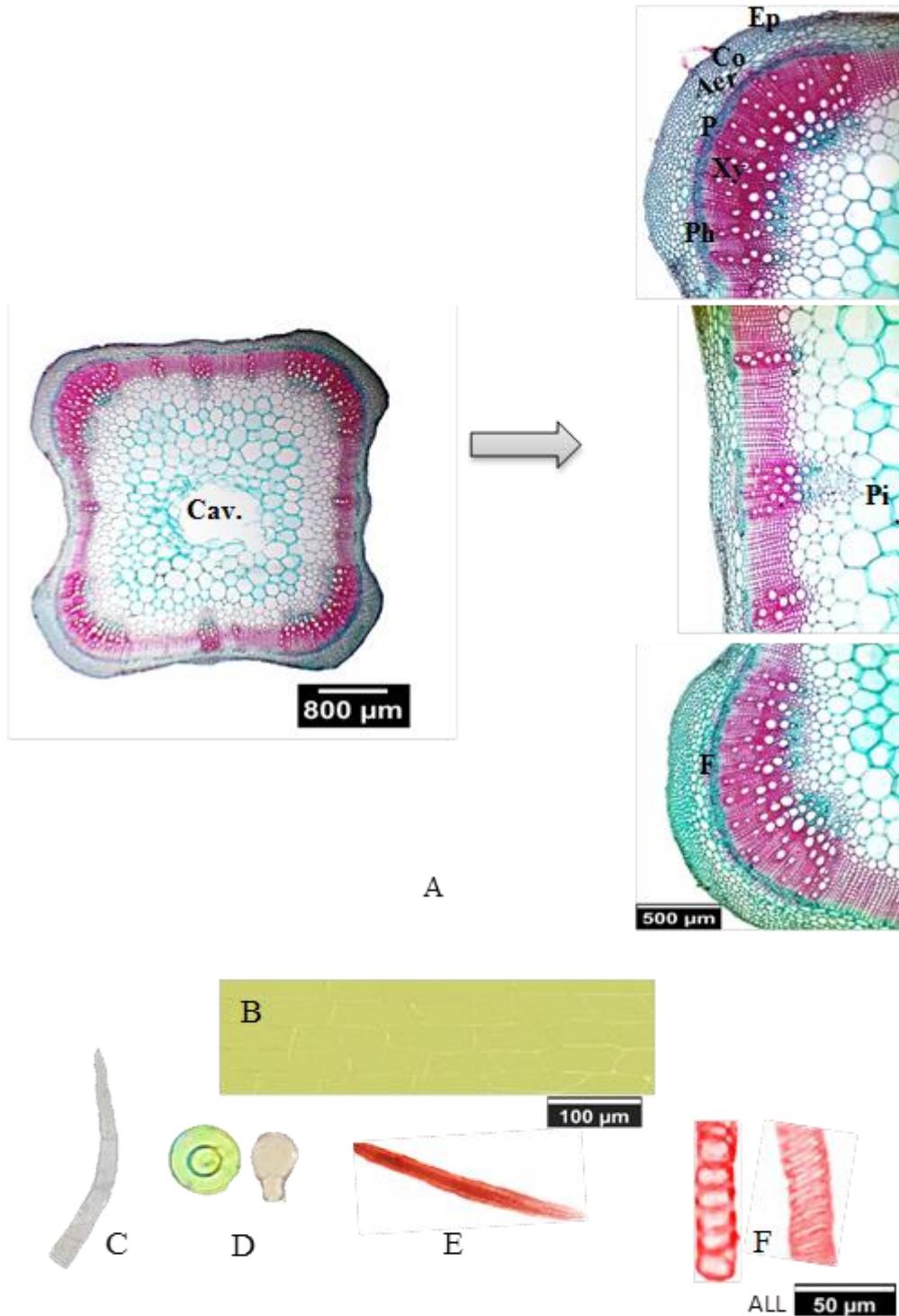
Fig. 1: Macro-morphology of *Salvia hispanica* L



A, Detailed transverse section of the leaf; *B*, Detailed transverse section of the petiole; *C*, upper epidermal cells of the lamina; *D*, lower epidermal cells of the lamina; *E*, upper neural epidermal cells; *F*, lower neural epidermal cells; *G*, upper epidermal cells of the petiole; *H*, lower epidermal cells of the petiole; *I*, diacytic stomata; *J*, non-glandular trichome; *K*, Glandular trichomes; *L*, starch granules; *M*, xylem vessels. (*A* x 100; *B* x 40; *C, D, E, F, G, H* x 400; *I, J, K, L, M* x 800)

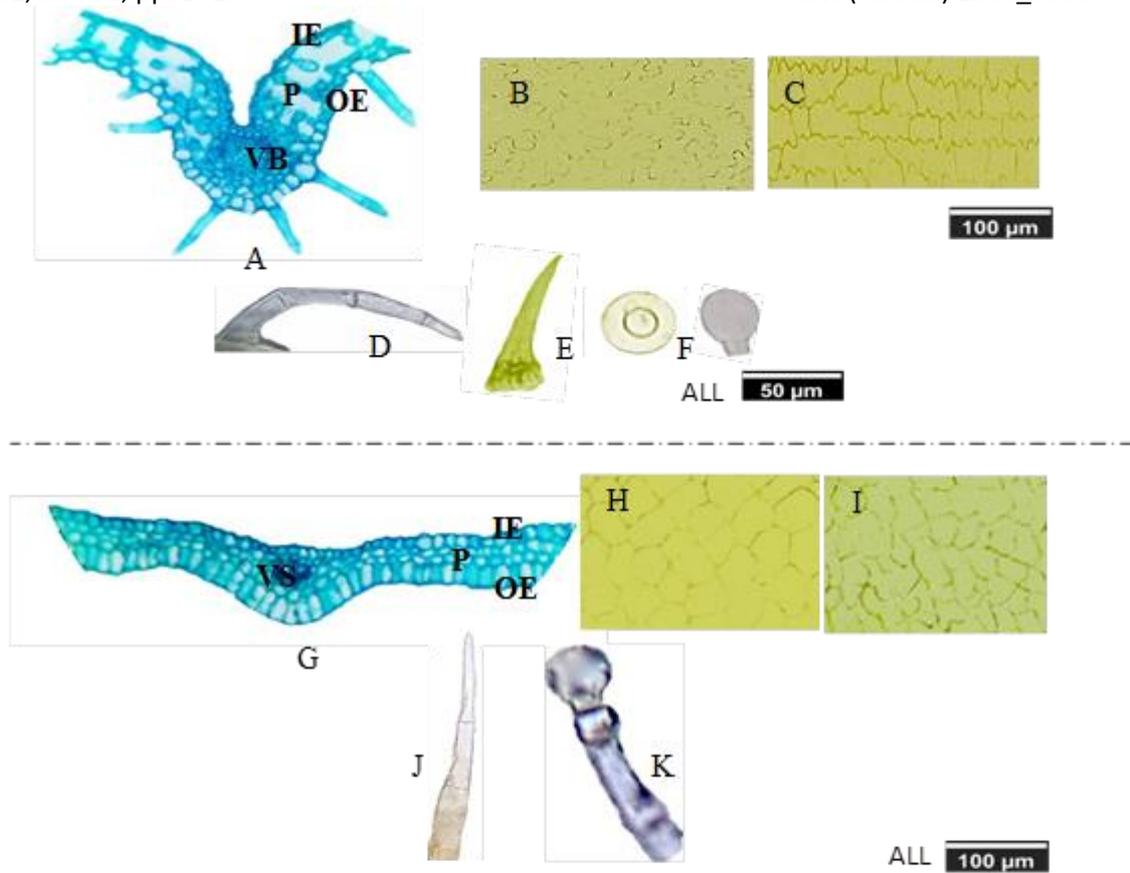
Co., Collenchyma; *LE.*, Lower epidermis; *Pal*, Palisade; *P.*, Parenchyma; *Sp.*, Spongy tissue; *UE.*, Upper epidermis; *VB.*, vascular bundles.

Fig. 2: The leaf and petiole



A, Detailed transverse section of the stem; *B*, epidermal cells of the stem; *C*, non-glandular trichome; *D*, Glandular trichomes; *E*, pericyclic fibre; *F*, xylem vessels (*Ax* 40; detailed *Ax* 100; *B* x 400; *C*, *D*, *E*, *F* x 800) *Aer.*, aerenchyma; *Cav.*, cavity; *Co.*, collenchyma; *Ep.*, epidermis ; *F.*, pericyclic fibre; *P.*, parenchyma; *Ph.*, phloem; *Pi.*, pith; *Xy.*, xylem

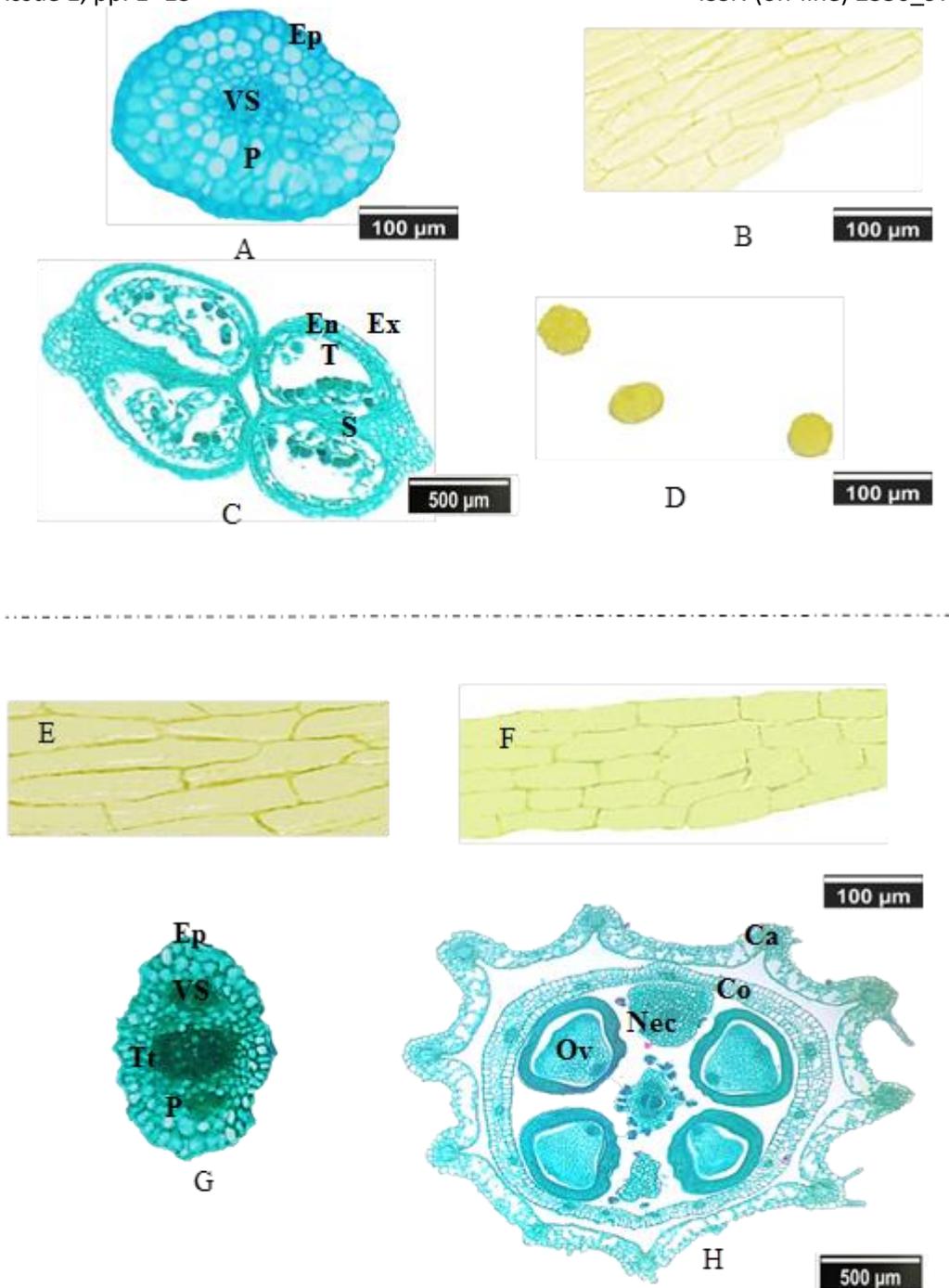
Fig. 3: The stem



A, transverse section of the calyx; B, outer epidermal cells of the calyx; C, inner epidermal cells of the calyx; D&E, non-glandular trichomes; F, glandular trichomes; G, transverse section of the corolla; H, outer epidermal cells of the corolla; I, inner epidermal cells of the corolla; J, non-glandular trichome; K, glandular trichome (All x 400 except D, E, F, G x 800)

IE., inner epidermis; OE., outer epidermis; P., Parenchyma; VB., vascular bundles; VS., Vascular strands

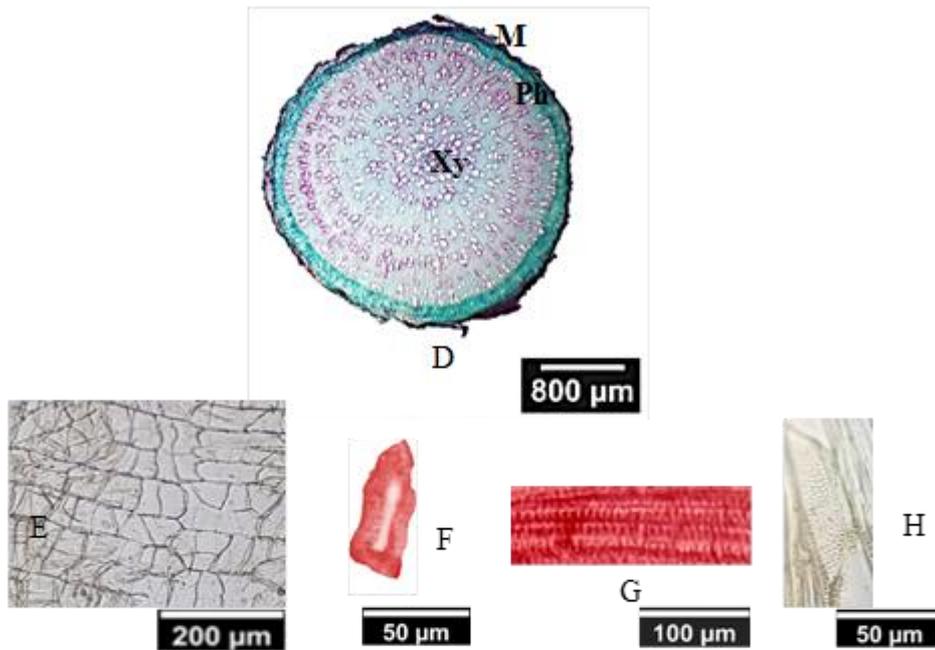
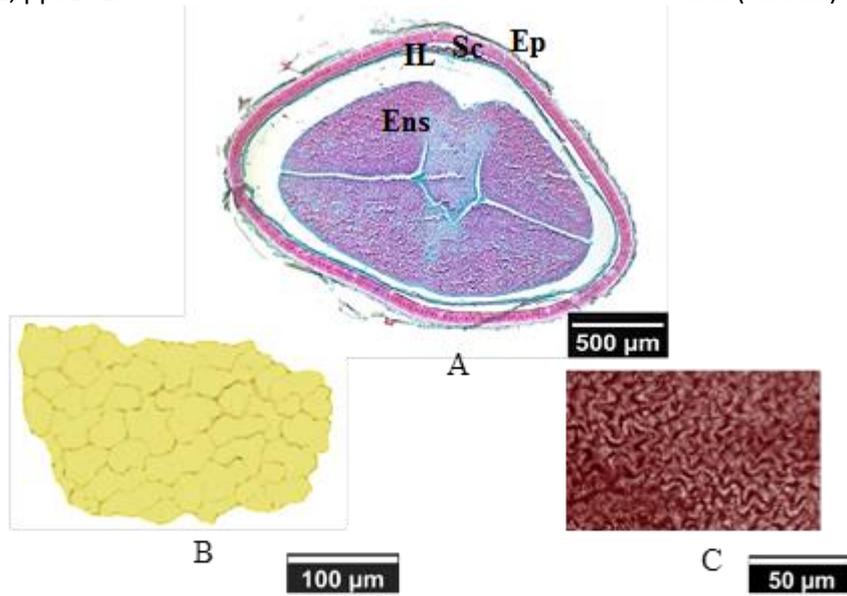
Fig. 4: The calyx and the corolla



A, transverse section of the filament; B, epidermal cells of the filament; C, transverse section of the anther; D, pollen grains; E, epidermal cells of the stigma; F, epidermal cells of the style; G, transverse section of the style; H, transverse section passing through the ovary (All x 400 except G, H x 100)

Ca., calyx; Co., corolla Ep., epidermis; En., endothecium; Ex., exothecium; Nec., nectary; Ov., ovules; P., Parenchyma; S., septum; T., tapetum; Tt., transmitting tissue; VS., Vascular strands

Fig. 5: The androecium and the gynoecium



A, transverse section of the seed; B, epidermal cells of the seed; C, seed coat sclerids; D, transverse section of the root; E, meta-derm cells; F, cortex sclerids; G, xylem vessels; H, tracheids (A x 100; B, G x 400; C, F, H x 800; D x 40; E x 200)

Ens, Endosperm; Ep., Epidermis; IL., inner layer of testa; M, Meta-derm; P., Parenchyma; Ph., Phloem; Sc., Sclerids; Xy., Xylem

Fig. 6: The seed and the root

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دراسة ظاهرية ومجهريية لنبات القيصين الإسباني المنزرع في مصر

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دراسة الخصائص الظاهرية والمجهريية لأوراق وعنق الورقة والسيقان والأزهار والبذور والجذور لنبات القيصين الإسباني حيث توطد هذه الدراسة سهولة التعرف على النبات وقد تم التعرف على وجود أنواع مختلفة من الشعيرات الغذائية والغير غذية وكذلك وجود طبقة من الصمغ المركز بخلايا البشرة لكل من الاوراق والبذور وأيضا أنواع مختلفة من الألياف والأوعية والعديد من الخلايا المميزة للنبات والعائلة التابع لها