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MORPHOLOGICAL AND ANATOMICAL STUDIES OF *Pimpinella anisum* L. (APIACEAE) III . ANATOMICAL STRUCTURE OF ROOT AND STEM

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ABSTRACT

This investigation was carried out to follow up the anatomical structure of the main root and stem of Anise throughout the consecutive stages of its entire life span .

The main root is of diarch protostele type .The secondary growth takes place in the common way when plants are 3 wks old. Formation of parenchymatous rays from the cambium opposite to the xylem ridges starts when plants are 6 wks old. In this stage, phellem is obvious. Schizogenous secretory canals occur in the pericycle . The secondary thickening is more prominent as plants are 12 wks old.

The anatomy of the internode directly below the axis of the compound umbel was studied at the age of 12 wks old as it represents the primary structure of the main stem. Surface of stem is strongly ridged and fluted. The ribs on the stem consist largely of collenchyma . The cortex in the grooves between the ridges consists of parenchyma of which the outer 3-4 layers are chlorenchyma . Secretory canals are embedded in the inner part of the cortex. The stele consists of 28-32 collateral vascular bundles arranged in a ring, 14-16 of them are large in size, lying on the radii which pass through the ridges. A large number of secretory canals are also found in the pith. The median internode of the main stem at the age of 12 wks is generally indifferent than that of the apical internode .

The basal internode of the main stem directly above the basal compact internodes at the age of 12 wks old is polygonal in outline. The ribs of the stem consist largely of sclerenchyma. The stele consists of 36-39 collateral vascular bundles distinct from one another except that the medullary rays are occluded at the xylem side of the ring forming conjunctive tissue of well lignified cells .

The stem basal portion of compact internodes 12 wks old is not ribbed , but it is cylindrical in outline . The cortex consists of 9-12 layers of compact parenchyma. Secondary thickening proceeds and secondary xylem is present in nearly a continuous cylindrical form. Anomalous secondary thickening occurs as numerous concentric bundles develop in the peripheral portion of the pith. Secretory canals are present in cortex , phloem rays and pith .

Key words : *anatomy , apiaceae , Pimpinella anisum L., root , stem .*

1. INTRODUCTION

The morphology of vegetative and reproductive growth of Anise plant (*Pimpinella anisum* L.) throughout the consecutive stages of its whole life span is given in the first two parts of this series of study (El-Sahhar *et al.*, 1999 a and b) . Consequently , it is aimed in this third part of the study to follow up the anatomical structure of the main root and stem of such species. Obviously , continued acquisition of new information about different botanical aspects of this species, which is of great interest from the economic and medicinal point of view, are required.

Metcalfe and Chalk (1979) stated that the root of *Pimpinella* , in the primary state of growth, is diarch. Endodermis with casparian thickening . Secretory canals are situated immediately within the epidermis , opposite both the phloem and xylem groups of the vascular strands. They also are present in the secondary phloem of older roots .

The same authors described , in general , the stems of umbelliferous plants and indicated that the stems of such plants are often ribbed, whilst the centre is occupied by a pith which often becomes hollow apart from the septa at the nodes. The ribs on the stems usually consist of collenchyma or, more rarely , of sclerenchyma. There is always a ring of vascular bundles , which may

be accompanied by medullary or , more rarely , cortical strands. Anomalous secondary thickening sometimes occurs in the stem . The anomalies include the development of numerous concentric bundles with central xylem .

Cronquist (1981) pointed out that stems of Apiaceae are commonly with well developed peripheral collenchyma , sometimes with cortical or pith bundles .

2. MATERIALS AND METHODS

The experimental work presented in this paper was carried out to follow up the anatomical structure of the main root and stem of Anise plant throughout the consecutive stages of its whole life span .

For this purpose , a field trial was conducted in the Experimental Station of the Faculty of Pharmacy , University of Cairo, Giza, throughout 1994 / 95 season to provide the experimental plant materials. The work of microtechnique was carried out at the laboratory of Agricultural Botany Department , Faculty of Agriculture, Cairo University , Giza .

The field trial included five replicates , each represented by one plot . The plot was 4x5 m with eight ridges 60 cm apart . Date of cultivation was October 29 th, 1994. Seeds were sown in hills spaced 20 cm. The plants were thinned to three plants per hill. All field practices were carried out as recommended for the plants in the vicinity .

Samples were taken fortnightly . A full microscopical study was carried out on specimens represented the main root through its basal portion and the main stem through terminal , median and basal portions. Microtechnique procedures given by Willey (1971) were followed. Materials 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 (melting point 56 - 58°C) . Transverse sections which were cut on a rotary microtome to a thickness of 20 microns were stained with safranin / light green before mounting in Canada balsam. Slides were examined microscopically and photomicrographed .

3. RESULTS AND DISCUSSION

3.1. Structure of the main root

The transverse section through the main root of two week old seedling of Anise (Fig.1) shows an obvious distinction among the three main tissue systems; *i.e.*, the epidermis (dermal tissue system) , the cortex (ground tissue system) , and the vascular tissue system. This justifies that the main root, at the age of two weeks, is in the primary state of growth. The epidermis consists of a single layer (uniseriate) of thin-walled cells lacked of a prominent cuticle layer and having the potentiality to form root hairs by a lateral extension of their outer walls. Underlying the epidermis is the cortical region (cortex), consisting of three to four layers of thin-walled irregular parenchymatous cells that are limited internally by a uniseriate endodermis which is regarded as a part of the cortex. The presence of triangular-intercellular spaces is characteristic of the cortical layers. The endodermis shows Casparian thickenings. Casparian strips are detected as dots in the transverse section. The pericycle consists of one layer following the endodermis to the inside and constitutes the outermost layer of the stele. The vascular cylinder is more compact and the stele is composed of two phloem strands, accompanied by a large amount of parenchyma cells, alternating with a similar number of exarch xylem ridges. Each xylem ridge comprised of some four vessels and the metaxylem vessels of the two ridges occupy the root centre forming a solid core. The pith is absent. Hence, the stele is regarded as a protostele; *i.e.*, the root is of diarch protostele type.

Secondary growth of the main root takes place in the common way. The cambium originates on the inner side of the phloem forming strands of two to three layers as a result of tangential divisions of the parenchymatous cells. When plants are three week old, 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 number of xylem vessels towards the inside and small amount of secondary phloem towards the outside. At the age of four weeks (Fig.2),the formation of secondary xylem and phloem proceeds. Fibres appear as small groups adjacent to the primary phloem. By formation of secondary xylem opposite the phloem, the cambium is

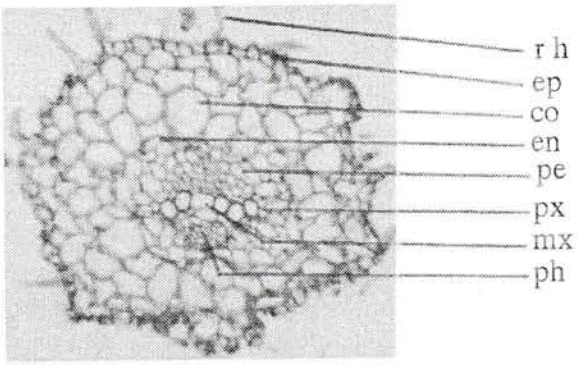


Fig. (1): Transverse section of two week old main root of *Pimpinella anisum* L., showing its primary structure. (X 144)

Details: co, cortex; en, endodermis; ep, epidermis; mx, metaxylem; pe, pericycle; ph, phloem; px, protoxylem and r h, root hairs.

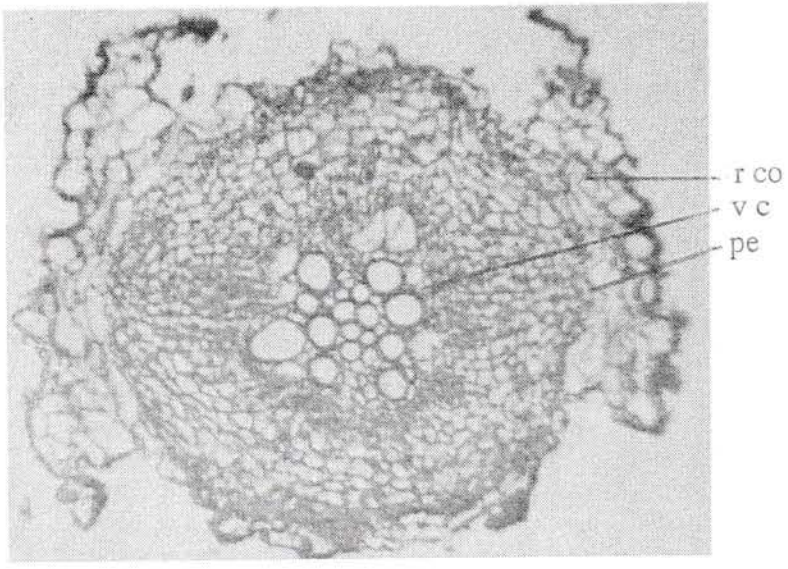


Fig. (2): Transverse section of four week old main root of *Pimpinella anisum* L., showing early stage of secondary growth. Notice: Secondary growth of vascular cylinder, cell division in pericycle, and rupture of cortex. (X 144)

Details: r co, ruptured cortex; pe, pericycle and v c, vascular cylinder.

moved outward, and eventually its circumference becomes circular in the transverse section. In the mean time, the cortex is ruptured at different regions (Fig.2) .

The transverse section through the main root of a six weeks old plant (Fig. 3 A and B) shows the formation of parenchymatous rays from the cambium originated in the pericycle opposite to the xylem ridges. Differentiation of the phellem from the phellogen is obvious in this stage of secondary growth to form the periderm (the protective layer). The pericyclic cells undergo periclinal and anticlinal divisions. The periclinal divisions cause an increase of the number of pericyclic layers in the radial extent. The combined increase in thickness of the vascular tissues and of the pericycle forces the cortex outward. The cortex does not undergo an increase in circumference, hence becomes ruptured and completely sloughed off together with the epidermis. Phellogen arises in the outer part of the pericycle and forms phellem (cork) toward the outside. Schizogenous secretory canals occur in the pericycle (Fig. 3 B) .

The secondary thickening is more prominent as tested plants were 12 week old (Fig. 4 A and B). The amount of secondary elements increases and the secondary xylem contains vessels of various diameters. The vessels are accompanied by fibres and parenchyma cells. Wide rays of lignified parenchyma divide the outer layers of the axial xylem into sectors in radial rows (Fig.4 B). The phloem contains sieve tubes with companion cells, fibres, and parenchyma cells. The wide rays of the xylem extend through the cambium and somewhat with similar rays through the phloem. Secretory canals are present in the secondary phloem. Cork derived from the phellogen forms the protective layer .

As far as the authors are aware, no detailed study dealing with the anatomical structure of Anise root was carried out. However, Metcalfe and Chalk (1979) stated that the root of *Pimpinella* is diarch. The endodermis shows Casparian thickenings. Pericyclic secretory canals are present in the secondary phloem of older roots. These results are in agreement with the present findings .

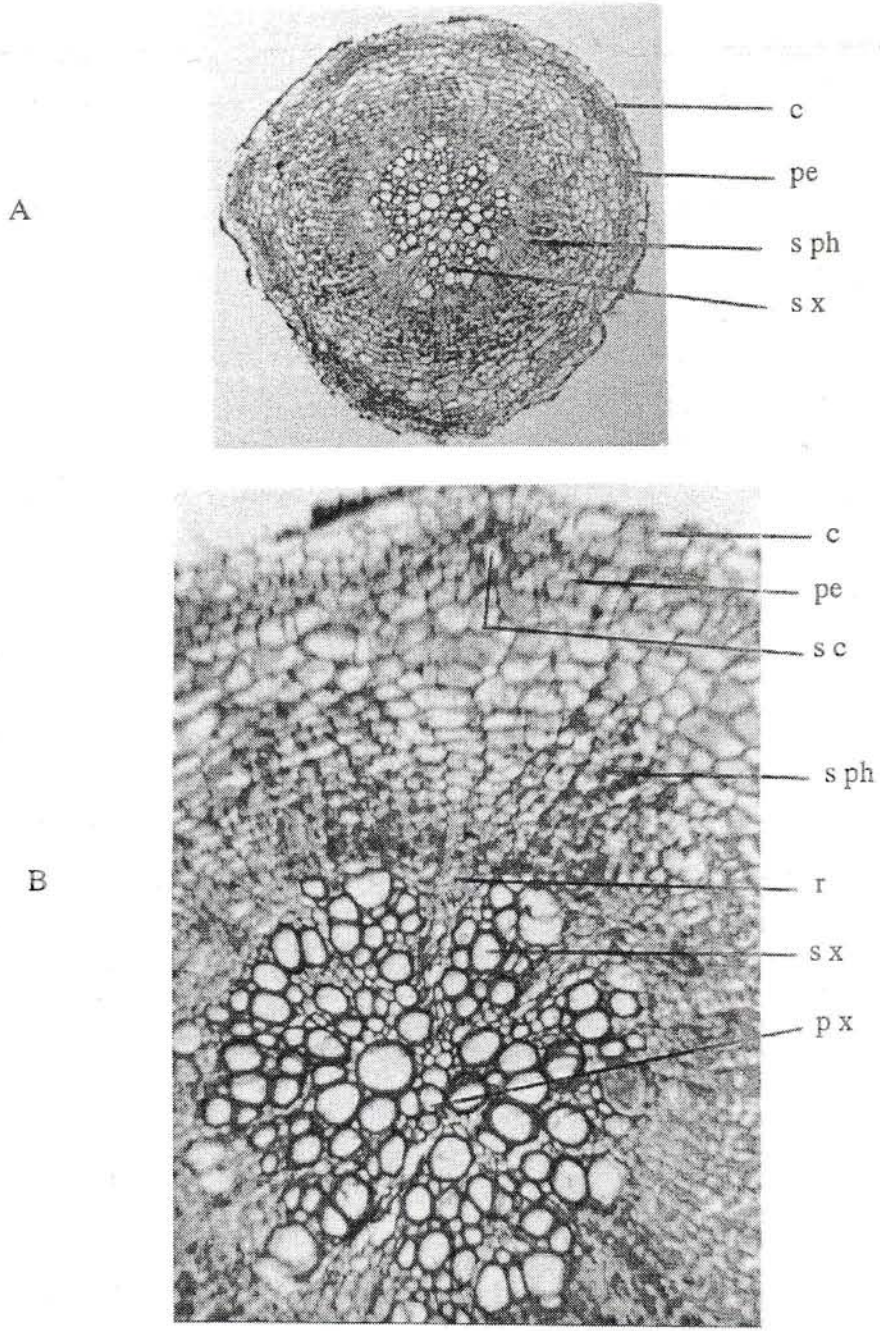


Fig. (3): Transverse section of the main root of *Pimpinella anisum* L., six week old, showing its secondary structure.
A: Whole section. (X 52)
B: Magnified portion of A. (X 144)

Details : c, cork; pe, pericycle; p x, primary xylem; r, ray; s c, secretory canals; s ph, secondary phloem and s x, secondary xylem.

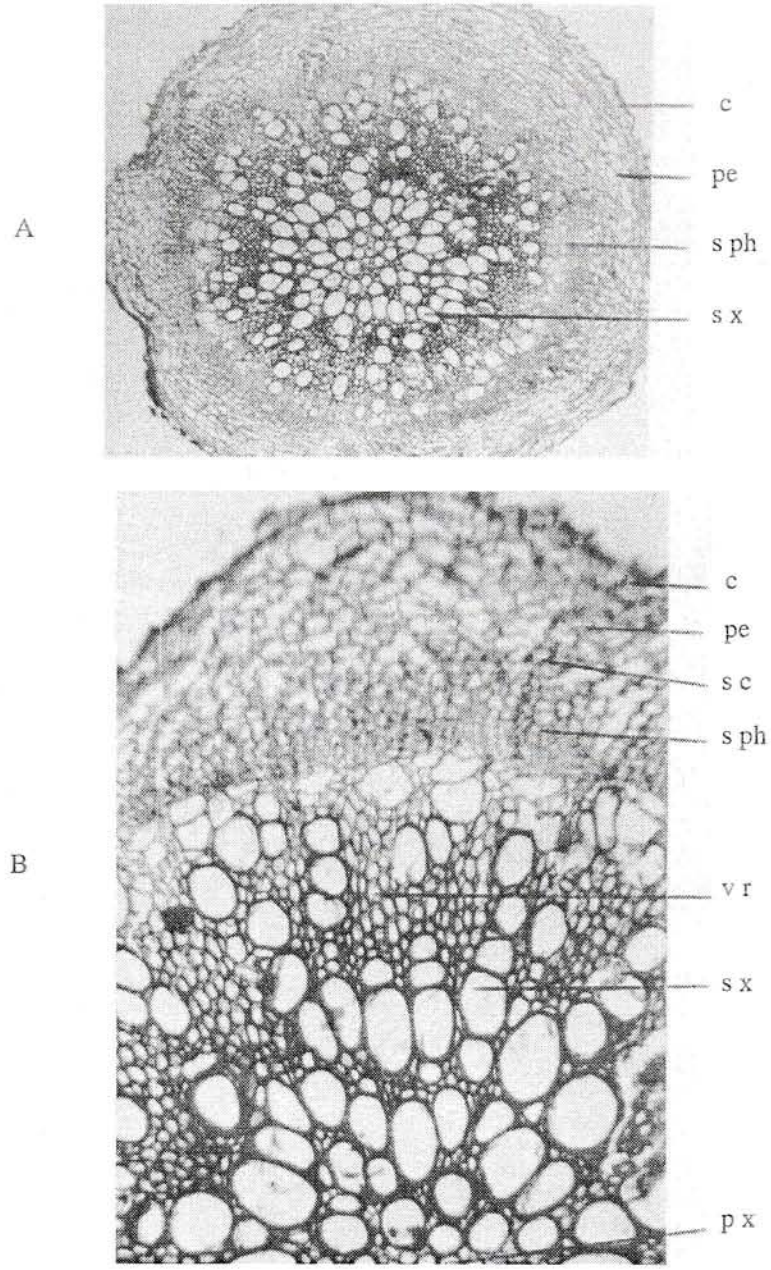


Fig. (4): Transverse section of the main root of *Pimpinella anisum* L., 12 week old, showing an advanced stage of secondary growth.

A: Whole section. (X 52)

B: Magnified portion of A. (X 144)

Details : c, cork; pe, pericycle; p x, primary xylem; s c, secretory canals; s ph, secondary phloem; s x, secondary xylem and v r, vascular ray.

3.2. Structure of the main stem

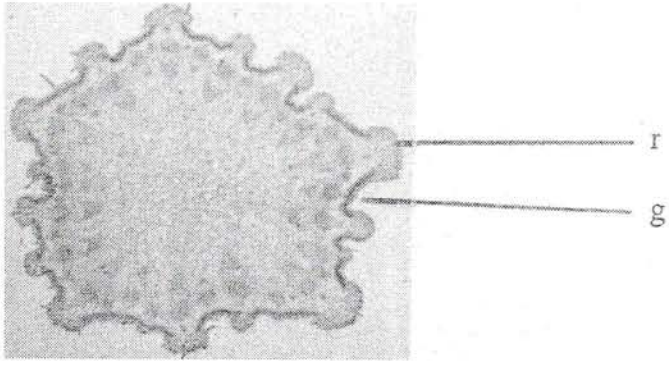
3.2.1. The apical internode

The internode directly below the axis of the compound umbel was studied from the anatomical point of view at the age of 12 weeks as it represents the primary structure of the main stem. The transverse sections shown in Figure (5) reveal that the stem surface of Anise plant is strongly ridged and fluted; *i.e.*, polygonal in outline. Epidermal cells are nearly square in shape and covered with a thin layer of cuticle. Stomata and bicellular trichomes are present in the epidermis. The ribs on the stem consist largely of collenchyma. It is evident that the strands of collenchyma constitute the chief mechanical tissue of the axis and form the ridges. The cortex in the grooves between the ridges consists of seven to eight layers of parenchymatous cells, the outer three or four layers are chlorenchyma underlying the epidermis. A part from the chlorenchyma that lies beneath the epidermis, except at the angles, and collenchyma occupying the ridges of the cortex, as stated earlier, the inner part of this region consists of a thin-walled compact tissue with secretory canals being embedded in it .

The vascular bundles are arranged in a ring and being separated from one another by medullary rays. The stele consists of 28 to 32 collateral bundles of which 14 to 16 are larger in size (major bundles) and the remainders are relatively small ones (minor bundles). The minor bundles are usually located in the region between any of two large ones . It is obvious that the major bundles are lying on the radii which pass through the stem angles (ridges). Each minor bundle has 9 to 13 vessels in 3 to 4 rows, while the major one has 27 to 33 vessels in nearly parallel 6 to 7 rows . It is worthy to note that the secretory canals which are present in the inner part of the cortex represent a characteristic feature of the stem structure and commonly occur outside the phloem of each vascular bundle.

The pith consists of polygonal parenchyma cells with relatively small intercellular spaces . A large number of secretory canals are also found in the pith (Fig. 5 D), most of them are located in the peripheral region of the pith . The pith is connected with the cortex by medullary rays 4 to 7 rows wide.

A



B

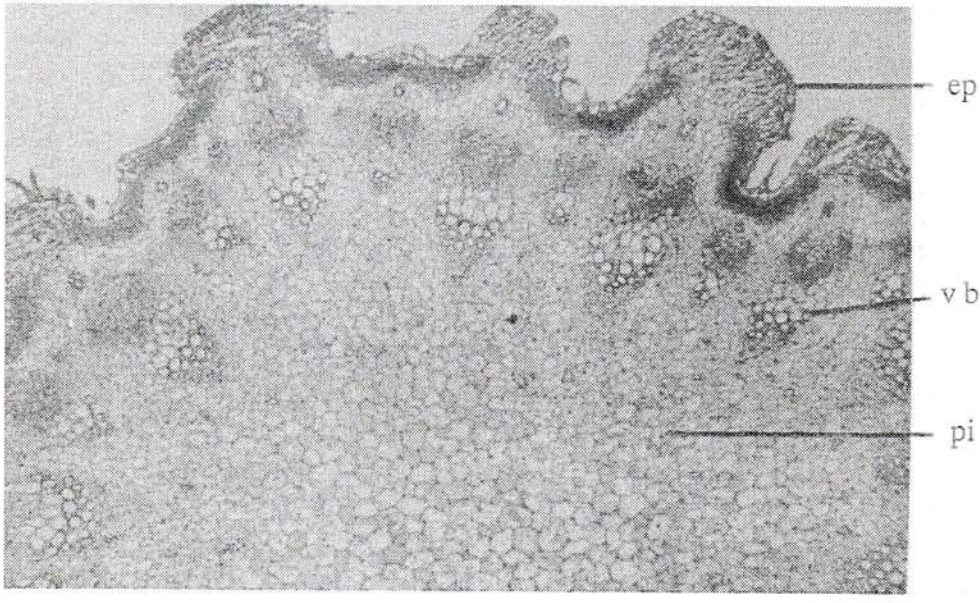


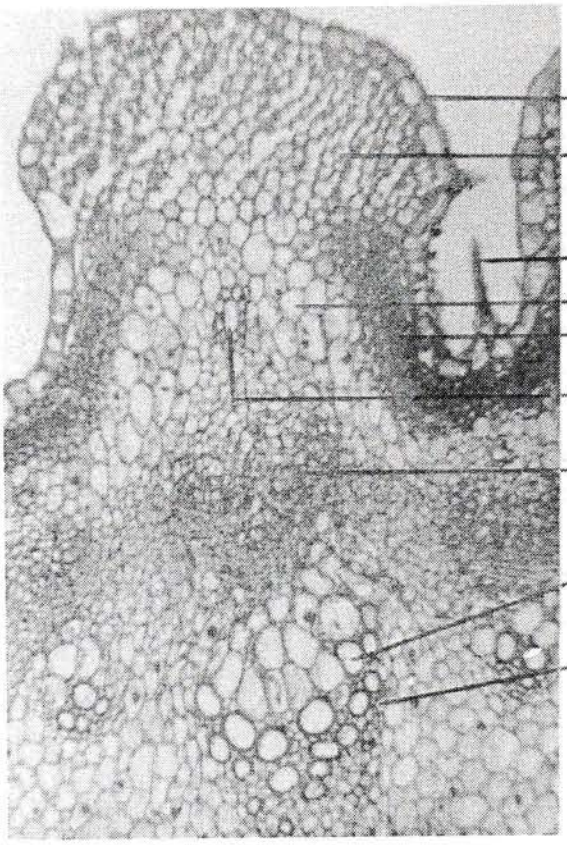
Fig. (5): Transverse section of the apical internode of the main stem of *Pimpinella anisum* L. at the age of 12 weeks.

A: Whole section. (X 20)

B: Magnified portion of A. (X 52)

(Cont.)

C



D

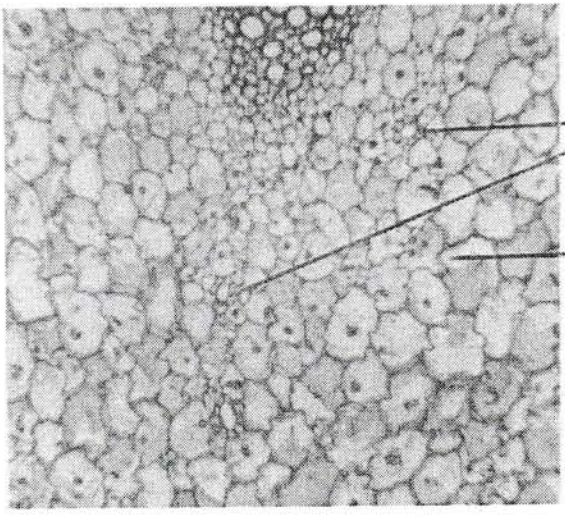


Fig. (5) : Cont.
C and D: Magnified portions of B. (X 144)

Details : ch, chlorenchyma; col, collenchyma; ep, epidermis; g, groove; pa, parenchyma; ph, phloem; pi, pith; r, ridge; s c, secretory canals; t, trichomes; v b, vascular bundles and x, xylem.

3.2.2. The median internode

The transverse sections through the median internode of the main stem of Anise plant at the age of 12 weeks are shown in Figure (6). Flowering stage starts when plants aged 12 weeks. At this age , the main stem attains its maximum length,being 50 to 55 cm. From the anatomical point of view, it is obvious that 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(Fig.5). The structure of the median internode is generally indifferent from that of the apical internode.The epidermis composed of a single layer contains stomata and trichomes (Fig.6 B). The outer walls of the epidermis are somewhat thickened and covered with a thin layer of cuticle . The ribs of the stem mainly consist of collenchyma. The collenchymatous strands at the ribs are oval or kidney- shaped in transection. The zone of chlorenchyma which lies beneath the epidermis, except at the angles, is two or three layers in width. The nonchlorophyllose parenchyma of the cortex (consists of 3 to 4 layers of large cells) occupies the region between the chlorenchyma and the vascular cylinder, also separating the collenchyma from the large bundles located at the angles of the stem. Stomata usually occur in the portions of the epidermis overlying the chlorenchyma. Secretory canals are formed in the inner part of the cortex, each opposite to the phloem of each vascular bundle .

The vascular tissues appear as a cylinder between the cortex and the pith. The vascular cylinder is separated by interfascicular regions into large and small collateral bundles in alternate arrangement. The number of larger bundles is nearly equal to that of the smaller ones, being 14 to 15.Each small bundle has 9 to 14 vessels in 3 to 4 rows, while the large one has 34 to 40 vessels in 6 to 8 rows. The pith consists of large polygonal parenchyma cells with relatively small intercellular spaces. It is worthy to note that secretory canals are formed also in the pith.

3.2.3. The basal internode

Transverse sections through the internode directly above the basal portion of the main stem of Anise plant at the age of 12 weeks are shown in Figure(7). It is clear that the stem is polygonal in outline. The epidermis composed of a single layer which include stomatas and

trichomes.Epidermal cells are nearly square in shape and covered with a cuticle layer. The ribs of the stem consist largely of sclerenchyma. Cortex at the distance between the angles comprised of 7 to 8 layers of parenchymatous cells of which the outer three layers are chlorenchyma underlying the epidermis. The non- chlorophyllose parencheyma of the cortex occupies the region between the chlorenchyma and vascular cylinder, also separating the sclerenchyma from the relatively large bundles located at the angles of the stem.

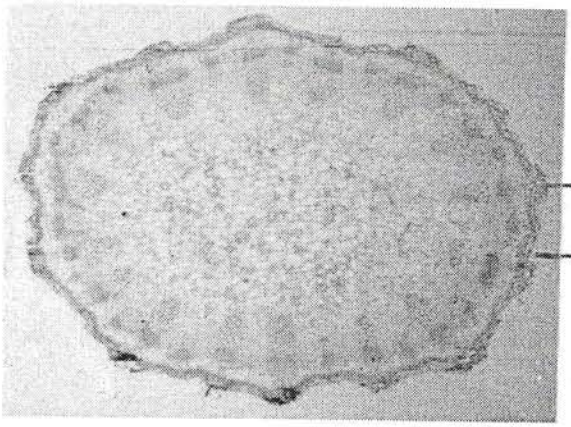
The vascular bundles are collateral and the stele consists of 36 to 39 bundles being relatively different in size. The large bundle has 64 to 70 vessels, while the intermediate bundle has 40 to 44 vessels and the small one has 17 to 21 vessels . The interfascicular cambium is absent. Therefore, the adjacent bundles are distinct from one another, except that the medullary rays are occluded on the xylem side of the ring. This may be due to the differentiation of the ray parenchyma to form connective or conjunctive tissue by thickening and lignification of cell walls as the stem matures. The fibres or connective type of cells also surrounds, to some extent, the inner face of the xylem of each bundle. The portion of the ray adjacent to the phloem does not mature as connective tissue , but the outer face of the phloem is capped by cells which tend to become thick- walled.

The pith consists of polygonal parenchyma cells with relatively small intercellular spaces. Secretory canals are present in the cortex, pith and sometimes are found in the conjunctive tissue.

3.2.4 The structure of the basal portion of the main stem

The basal portion of the main stem of Anise plant, comprised of four basal internodes which remain compact and are not distinguished throughout the whole life of the plant.The structure of compact internodes may differ than that of the normal ones. It was examined at the age of 12 weeks in form of transverse sections (Fig.8). It is obvious that the basal portion is not ribbed as the remainder of the main stem, but it is cylindrical in outline. The epidermis consists of one layer, the epidermal cells are relatively small in size, nearly square in shape and covered with a thin cuticle layer; trichomes are not observed. The cortex consists of 9 to 12 layers, all of compact parenchyma cells. The mechanical tissue, either collenchyma or sclerenchyma , and chlorenchyma are absent from the cortex.

A



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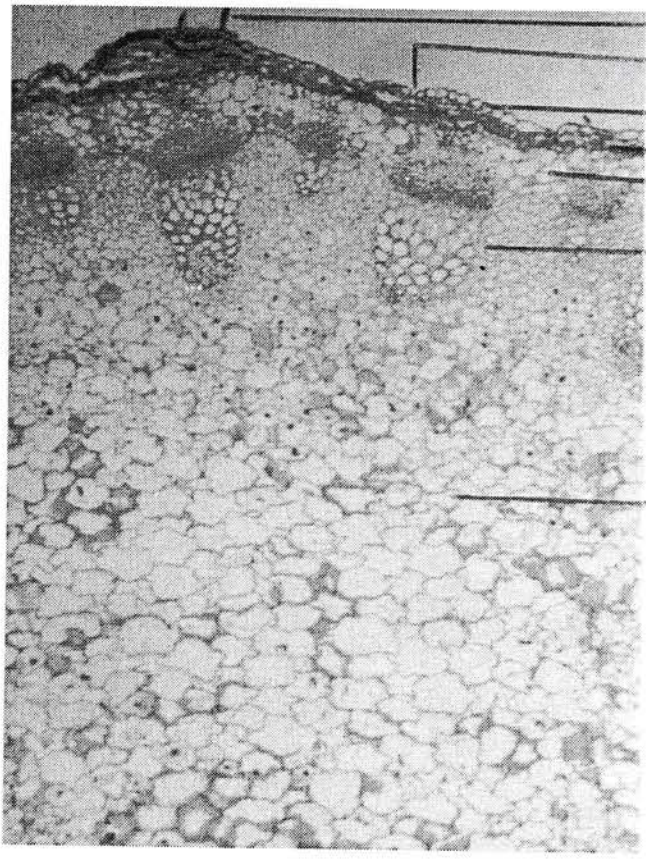

g. (6): Transverse section of the median internode of the main stem of *Pimpinella anisum* L. at the age of 12 weeks.

A: Whole section. (X 20)

B: Magnified portion of A. (X 52)

Details : ch, chlorenchyma; ep, epidermis; f, furrow; pa, parenchyma; pi, pith; r, rib; st, stoma; t, trichomes and v b, vascular bundles.

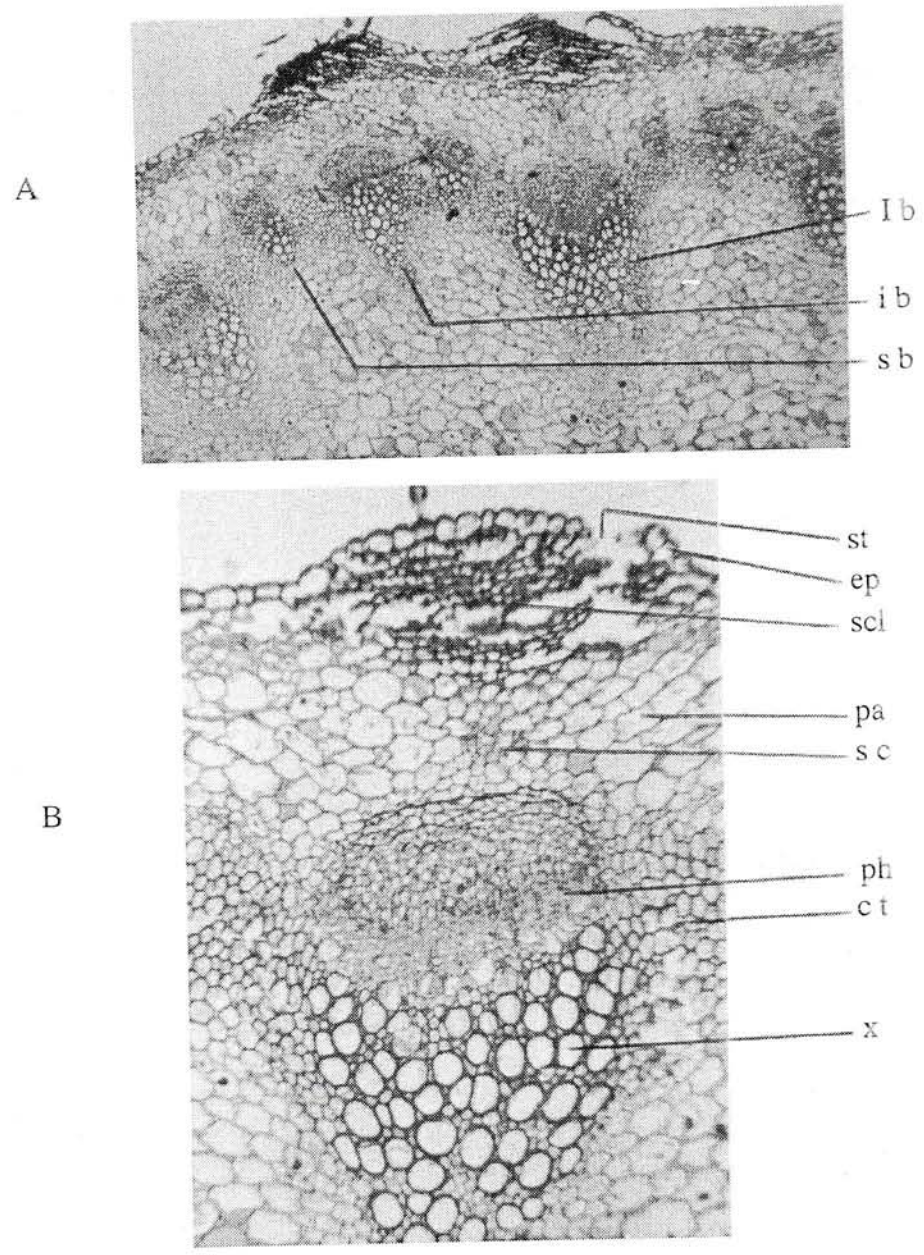

Fig. (7): Transverse section of the basal internode of the main stem of *Pimpinella anisum* L. at the age of 12 weeks.
A: A portion of the section. (X 52)
B: Magnified portion of A. (X 144)

Details: c t, conjunctive tissue; ep, epidermis; i b, intermediate bundle; l b, large bundle; pa, parenchyma; ph, phloem; s b, small bundle; s c, secretory canal; scl, sclerenchyma; st, stomata; and x, xylem.

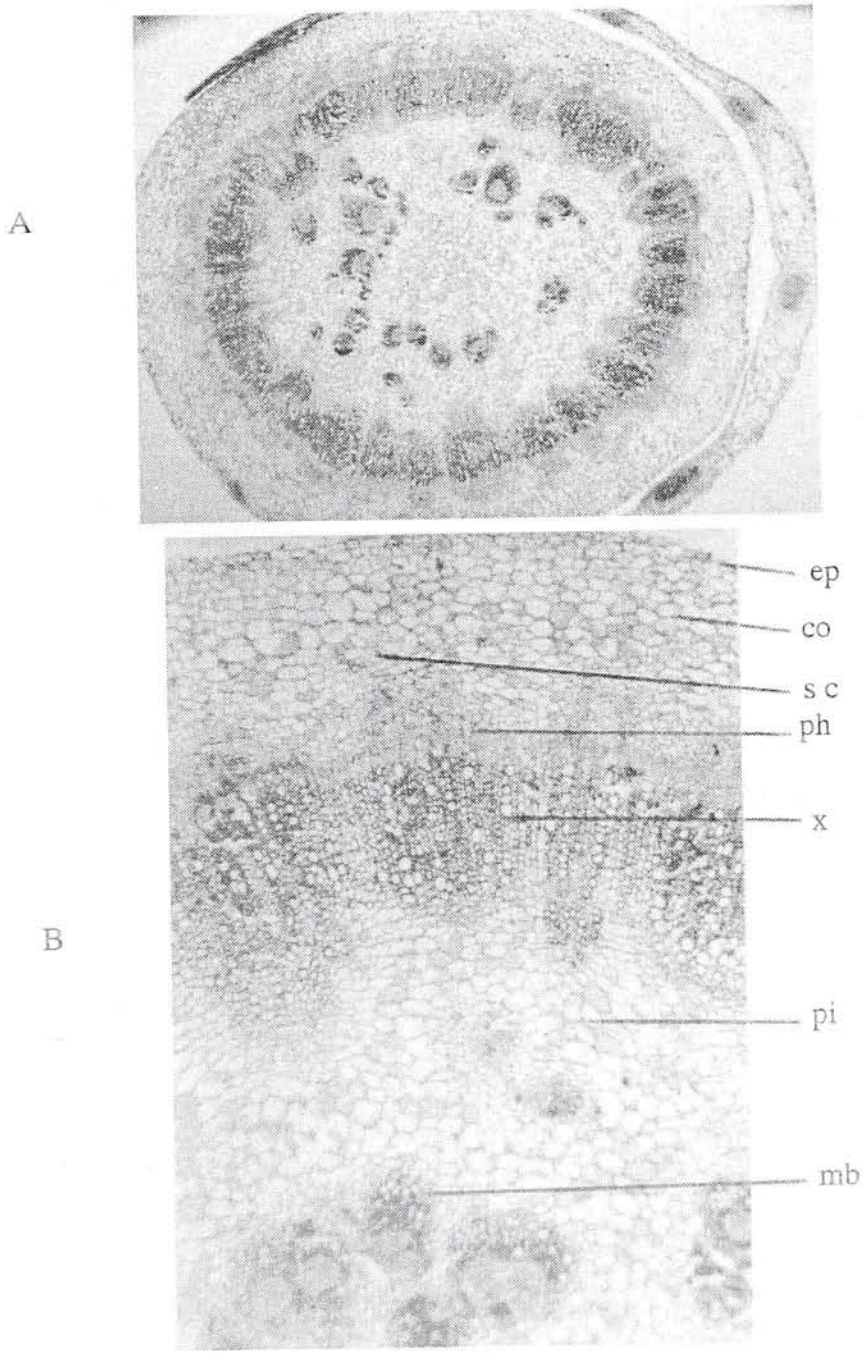

Fig. (8): Transverse section of the basal portion of the main stem of *Pimpinella anisum* L. at the age of 12 weeks.
A: Whole section. (X 20)
B: Magnified portion of A. (X 52)

Details: co, cortex; ep, epidermis; m b, medullary bundles; ph, phloem; pi, pith; s c, secretory canal and x, xylem.

The vascular cylinder is composed of collateral bundles. Secondary thickening proceeds and secondary xylem is present in nearly a continuous cylindrical form consisting of vessels arranged in radial rows embedded in lignified parenchyma cells. The primary xylem is recognized abutting the pith.

Anomalous secondary thickening occurs in the basal portion of the main stem. The anomalies include the development of numerous concentric bundles in the peripheral portion of the pith due to the formation of extrafascicular cambial rings which produces phloem on the inside and xylem on the outside . In most examined sections, the phloem is well developed , while the extent of xylem formation is variable. Therefore, the medullary bundle may be completely amphivasal or half-amphivasal.

It is worthy to note that secretory canals are present in cortex, phloem rays and pith.

3.2.5. Trichomes

The epidermis of Anise stem forms trichomes of nonglandular hairs which may be bicellular or uniseriate composed of three cells of which the terminal cell tapers .Some of the bicellular hairs are nearly conical in shape and some others seem to be sharp like spine (Fig.5 C).

As far as the authors are aware, no detailed study dealing with the anatomical structure of Anise stem was carried out. However,Metacalfe and Chalk (1979) indicated that the stems of *Pimpinella* are often ribbed . The ribs on the stems usually consist of collenchyma or, more rarely, of sclerenchyma .There is always a ring of vascular bundles, which may be accompanied by medullary or, more rarely, cortical strands. Secretory canals present in the inner part of the cortex and at the periphery of the pith. Anomalous secondary thickening sometimes occurs in the stem.These results are in accordance with the present findings .

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دراسات مورفولوجية وتشريحية على نبات الينسون من الفصيلة الخيمية
٣- التركيب التشريحى للجذر والساق

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قسم النبات الزراعى ــ كلية الزراعة ــ جامعة القاهرة

ملخص

أجرى هذا البحث لمتابعة التركيب التشريحى لجذر وساق نبات الينسون خلال المراحل المتتالية لدورة حياته.

كان العمود الوعائى للجذر الرئيسى من طراز العمود الوعائى الأول ثنائى أذرع الخشب . يحدث النمو الثانوى بالطريقة المعتادة عند عمر ٣ أسابيع . تتكون الأشعة البارنشيمية من الكامبيوم الناشىء مقابل أذرع الخشب عند عمر ٦ أسابيع ، يكون الفلين فى هذه المرحلة واضحاً ، كما يلاحظ وجود القنوات الإفرازية الإنفصالية فى البريسيكل. يصبح النمو الثانوى للجذر الرئيسى أكثر وضوحاً عند عمر ١٢ أسبوعاً.

تم دراسة التركيب التشريحى للسلامية التى تقع أسفل محور النورة المركبة للساق الرئيسية مباشرة عند عمر ١٢ أسبوعاً حيث تمثل التركيب الإبتدائى للساق

الرئيسية. وقد وجد أن سطح الساق الرئيسية عند هذه السلامية يكون مضلعاً ومحززاً بشدة . تتركب أضلاع الساق أساساً من الكولنشيمة ، وتتكون القشرة في مناطق الأخاديد التى بين الأضلاع من البارنشيمة حيث تصبح الثلاث أو الأربع طبقات الخارجية منها كلورنشيمية . وتوجد قنوات إفرازية مطمورة بالجزء الداخلى من القشرة. يتكون العمود الوعائى من ٢٨ــ ٣٢ حزمة وعائية جانبية مرتبة فى حلقة منها ١٤ــ ١٦ حزمة وعائية كبيرة الحجم تقع على الأقطار التى تمر خلال زوايا الساق . يوجد أيضاً عدد كبير من القنوات الإفرازية فى النخاع . تتماثل السلامية الوسطية للساق الرئيسية عند عمر ١٢ أسبوعاً من الناحية التشريحية مع السلامية الطرفية.

تبدو السلامية القاعدية للساق الرئيسية التى تقع أعلى المنطقة القاعدية المنضغطة مباشرة عند عمر ١٢ أسبوعاً عديدة الأضلاع . تتركب أضلاع الساق أساساً من اسكلرنشيمة. يتكون العمود الوعائى من ٣٦ــ ٣٩ حزمة وعائية جانبية تتميز عن بعضها البعض فيما عدا تلك التى بالأشعة النخاعية التى تسد جانب الخشب من حلقة الحزم الوعائية مكونة نسيج إتصال من خلايا ملجننة بشدة.

تتكون المنطقة القاعدية للساق الرئيسية عند عمر ١٢ أسبوعاً من سلاميات منضغطة إسطوانية فى شكلها الخارجى وتشتمل القشرة على ٩ ــ١٢ طبقة من خلايا بارنشيمية منضغطة . يحدث النمو الثانوى ويظهر الخشب الثانوى بشكل اسطوانة متصلة تقريباً ، كما يحدث نمو ثانوى شاذ حيث يتكشف العديد من الحزم الوعائية المركزية فى المنطقة المحيطية للنخاع . وتوجد قنوات إفرازية فى كل من القشرة وأشعة اللحاء والنخاع.

المجلة العلمية لكلية الزراعة ــ جامعة القاهرة ــ المجلد (٥٢) العدد الرابع (أكتوبر ٢٠٠١):٥٣٧-٥٥٦.