MORPHOLOGICAL AND ANATOMICAL STUDIES OF Pimpinella anisum L. (APIACEAE) IV . ANATOMICAL STRUCTURE OF LEAVES , FLOWER BUDS AND FRUITS

(Received: 19.2.2001)

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ABSTRACT

Anatomical structure of leaves, flower buds and fruits of Anise plant was investigated. Blades of the third and the eighth leaf on the main stem as well as the leaves on lateral branches nearly have the same structure. Leaves are dorsiventral. Compact arrangement of epidermal cells and presence of cuticle layer, anisocytic stomata and trichomes are the main features of the Anise leaf epidermis. At the midrib region, both upper and lower epidermis are convex and covered with a thick layer of cuticle especially at the abaxial surface. Trichomes are present on both surfaces and they are of unicellular, bicellular or uniseriate type composed of three cells. The palisade tissue consists of two layers of cells which occupy one - half of the whole thickness of the mesophyll. Vascular bundles of the principal veins are accompanied, from above and below, by a parenchyma tissue in which a secretory canal lies above (small one) and below (large one) the vein. The bundle is sometimes surrounded by a sheath of one layer of parenchymatous cells .

The petiole is almost pentagonal in outline, bounded by an uniseriate epidermis of nearly square-shaped cells. Stomata and trichomes are present. The angles, beneath the epidermis, consist mainly of sclerenchyma. The ground tissue consists mostly of relatively large

parenchyma cells, except at the distance between the angles where there are 2-3 layers of chlorenchyma cells directly underlying the epidermis. The vascular tissues are formed of five main collateral bundles which are arranged in crescent shape, and separated from one another by wide spaces of ground tissue. Secretory canals are embedded in the parenchymatous ground tissue, each opposite to the phloem of every vascular bundle.

The sheath of leaf is nearly crescent in shape and bounded by a uniseriate epidermis. Stomata are present, but trichomes are rarely found. There are five collateral vascular bundles, embedded in the ground tissue, different in size and arranged in crescent shape. Secretory canals are present in the ground tissue at the tapering ends of the sheath and opposite to the phloem of each bundle.

As to the floral buds, the sepals of calyx are absent. Corolla consists of five incurved petals. The epidermal cells of the petal develop trichomes. At the centre of each petal, there is one small vascular bundle embedded in the ground tissue and an obvious secretory canal opposite to the phloem of such bundle. The stamens are five, alternate with the petals. The stamen consists of a two-lobed four-loculed anther borne on the filament, a thin stalk with a single vascular bundle. Gynoecium is comprised of two united carpels. The ovary is bilocular, and in each loculus a single anatropous pendulous ovule which hangs from the median septum, placentation is apical. Ovary prominently ridged, with obvious secretory canals (vittae), and covered with numerous short trichomes. Each carpel usually has five ridges.

The fruit consists of two mericarps of which one is fertile (develops one fertile seed) and the other mericarp is usually sterile. Each mericarp has two, sometimes three, large vittae on the commissural surface and about twenty to thirty small vittae on the dorsal surface. Between the vittae, the pericarp is ridged externally and a vascular strand is contained in each of these ridges. The fruit is orthospermous. The seed is attached by its testa to the pericarp (schizocarpic fruit) so that it completely fills the loculus. The seed contains a small dicotyledonous embryo at the apical end, embedded in an abundant oily endosperm.

Key words: anatomy, apiaceae, bud, flower, fruit, leaf, Pimpinella anisum.

1. INTRODUCTION

The anatomical structure of the main root and stem of Anise plant was carefully investigated in the third part of this series of study (Nassar et al., 2001). Moreover, it is aimed in this part of the study to follow up the anatomical structure of foliage leaves, flower buds and fruits of the same plant in order to complete the anatomical map of such important species of Apiaceae.

1.1. Leaf

Metcalfe and Chalk (1979) pointed out that the leaf of umbelliferous plants is usually dorsiventral, except in species which show ecological specializations. The hairs, which are nearly always non-glandular, include unicellular, dendroid and stellate types. Secretory canals, which contain a mixture of oils, resin, and mucilage, are a particularly characteristic feature. They are present in the petiole and leaf lamina. The petiole is usually provided with an arc or ring of vascular bundles.

Cronquist (1981) mentioned that leaves of Apiaceae have stomata of various types, most often paracytic or anomocytic or anisocytic. Petiole commonly with a ring or arc of vascular bundles, sometimes with medullary bundles as well.

1.2. Flower

Fitting et al., (1930), Bailey (1969) and Radford et al., (1974) described the inflorescence of umbelliferous plants as terminal, frequently over topped by the next younger lateral shoot. It is an umbel, or more frequently a compound umbel, the secondary being known as umbellets, the bracts forming the involucre and partial involucres, or an involucre may be wanting. The umbellets subtended by bractlets forming the involucels. Flowers small, usually bisexual, epigynous, white, greenish, or yellow; other colours are rare.

Floral formula : O, O, CA_5 , CO_5 , A_5 , $G_{(2)}$.

The sepals are usually represented by short teeth or wanted. The flowers at the circumference of the compound umbel sometimes become zygomorphic by the enlargement of the outwardly directed

petals. Ovary is inferior, always bicarpellary and bilocular; in each loculus a single ovule hangs from the median septum with its micropyle and is directed upwards and outwards. The upper surface of the carpels is occupied by a swollen, nectar-secreting disc continuing into the longer or shorter styles, which terminate in spherical stigmas.

Hutchinson (1979) mentioned that the flowers of Apiaceae are usually bisexual, rarely unisexual, in simple or compound umbels or rarely capitate; calyx adnate to the ovary, 5-lobed; petals 5 valvate or slightly imbricate, epigenous, free, soon falling off, mostly inflexed in bud; stamens 5, alternate with the petals; filaments inflexed in bud; anthers 2-locular, opening lengthwise; ovary inferior, 2-locular; styles 2, thickened at the base and capping the ovary; ovules solitary in each loculus, pendulous.

1.3. Fruit

Wallis (1967) stated that the fruit of Anise is orthospermous; *i.e.*, the seed is flat on the inner or ventral surface. Mericarp with 30 to 40 vittae on the dorsal surface, epidermis with short, stiff trichomes. Anise fruits occur usually as entire mericarps with the pedicels attached. The mericarp is about 3 to 5 mm long and 1.5 to 2 mm. wide; it is ovoid - conical, greyish - brown and rough to the touch, owing to the presence of numerous short, conical epidermal trichomes 20 to 160 μ long and 15 to 40 μ wide at the base; it is crowned by a short, bifurcate stylopod. Each mericarp has two, sometimes three or four large vittae on the commissural surface and about 20 to 40 small vittae on the dorsal surface. The large number of vittae has arisen by the branching of four original ducts. These results are in harmony with those given by Fitting *et al.* (1930) and Parry (1945).

2. MATERIALS AND METHODS

The present study was carried out to investigate the histological structure of different types of foliage leaves, flower buds and mature fruits of Anise plant (*Pimpinella anisum* L.)

Therefore, a field trial was conducted in the Experimental Station of the Faculty of Pharmacy, University of Cairo, Giza

throughout 1994 /1995 season to provide the experimental plant materials. The work of microtechnique was carried out at the laboratory of the Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza during the two successive years of 1995 and 1996.

The field trial included five replicates, each represented by one plot. The plot was 4×5 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 representing the following organs:

1-Different types of foliage leaves developed on the main stem and lateral branches represented by the middle of the lamina, petiole and sheath.

2-Flower buds and mature fruits.

Microtechnique procedures given by Willey (1971) were followed. Materials were killed and fixed for at least 48 hr 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 μ were stained with saffranin / light green before mounting in Canada balsam. Slides were examined microscopically and photomicrographed .

3. RESULTS AND DISCUSSION

3.1. Structure of the leaf

3.1.1. Leaf blade (lamina)

Anise plant bears different foliage leaf types. The four to five basal foliage leaves are simple and the others upward are compound where the leaf is divided into leaflets. The compound leaves differ according to their position on plant. Leaf number 5 and 6 are trifoliate and leaf number 7 to 12 is imparipinnately compound (leaflets five to seven in number). Leaflets of leaf numbers 5 to 12 resemble the blade of simple leaf on the lower portion of the shoot. While, leaflets

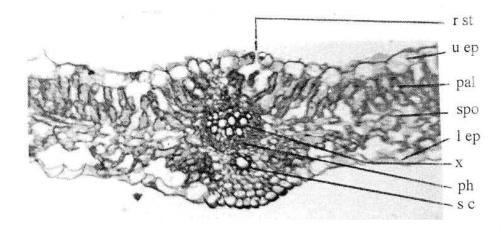
of leaf number 13 and upward, including all those on the lateral branches, are parted into linear-lanceolate segments and the leaf being finely divided feathery pinnate.

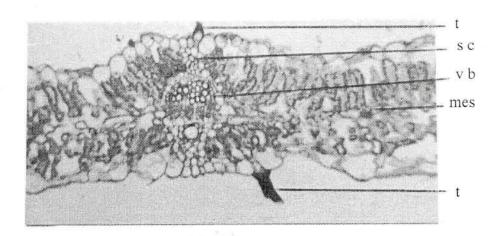
Therefore, the anatomical structure of leaf blades representing all different types of foliage leaves were investigated. Transverse sections of leaf number 3, 8 and of lateral branches were examined. It was found that all these leaves nearly have the same structure. Leaves are dorsiventral; i.e., the palisade tissue is located on the adaxial side of the blade and the spongy tissue on the abaxial one (Figs. 1 and 2). Compact arrangement of epidermal cells and the presence of cuticle layer, stomata and trichomes are the main features of the Anise leaf epidermis. Stomata occur on both sides (surfaces) of the leaf. The upper surface seems to be undulate and stomata are located above the surface of the epidermis; i.e.,raised stomata (Fig. 1), it is of Anisocytic type (Fig.3). The outer walls of the epidermal cells are slightly thickened, where the epidermis overlies the chlorophyll parenchyma, and is very much thickened over the veins.

At the midrib region, both upper and lower epidermis are convex and covered with a thick layer of cuticle especially at the abaxial surface. Trichomes are present on both surfaces, they are of unicellular, bicellular or uniseriate type composed of three cells(Figs. 1 B and 2). The palisade tissue consists of two layers of cells elongated perpendicularly to the surface of the blade being characterized by an abundance of chloroplasts. The palisade tissue occupies one-half of the whole thickness of the mesophyll. In addition, the spongy tissue is composed of 2 to 3 layers of chlorenchymatous loosely arranged cells with many wide intercellular spaces.

Vascular bundles of the principal veins are accompanied from above and below by a parenchyma tissue in which a secretory canal lies above (small one) and below (large one) the vein. The vascular bundle is oriented with the xylem directed towards the adaxial surface and the phloem towards the abaxial one. The bundle is sometimes surrounded by a sheath of one layer of parenchymatous cells (Fig.1 B).

The petiole of Anise leaf as seen in the transverse section (Fig. 3.1.2. Leaf petiole 4) is almost pentagonal in outline, bounded by a uniseriate epidermis of nearly square-shaped cells. The outer walls of the epidermis are





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Fig.(1): Transverse sections through a leastlet from leaf number eight on the main stem of *Pimpinella anisum* L. at the age of 12 weeks.

A: Transverse section through the midvein. (X 144)
B: Transverse section through the marginal portion. (X 144)

Details: l ep, lower epidermis; mes, mesophyll; pal, palisade tissue; ph, phloem; r st, raised stomata; s c, secretory canal; spo, spongy tissue; t,

trichomes; u ep, upper epidermis; v b, vascular bundle and x, xylem.

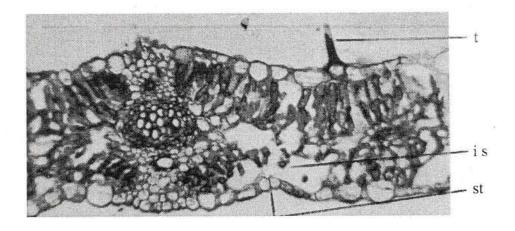


Fig.(2): Transverse section through a leaslet from leaves developed on the lateral branches of *Pimpinella anisum* L. at the age of 14 weeks. (X 144)

Details: i s, intercellular spaces; st, stomata and t, trichomes.

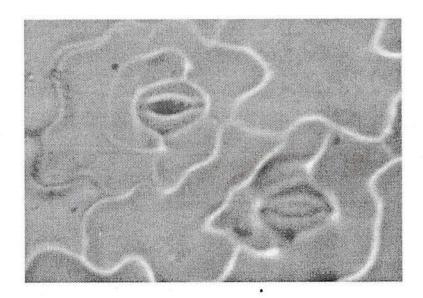


Fig.(3'): Epidermal peel showing stomata in leaslet of *Pimpinella anisum* L., being of Anisocytic type. (X 570)

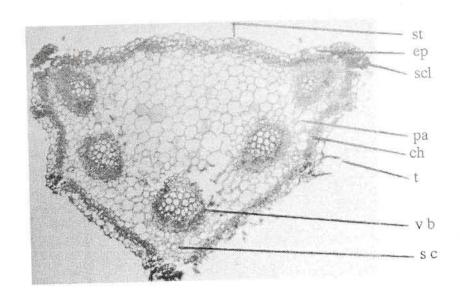


Fig. (4): Transverse section through the petiole of the eighth leaf on the main stem of Pimpinella anisum L. (X 52)

Details: ch, chlorenchyma; ep, epidermis; pa, parenchyma; s c, secretory canal; scl, sclerenchyma; st, stomata; t, trichomes and v b, vascular bundle.

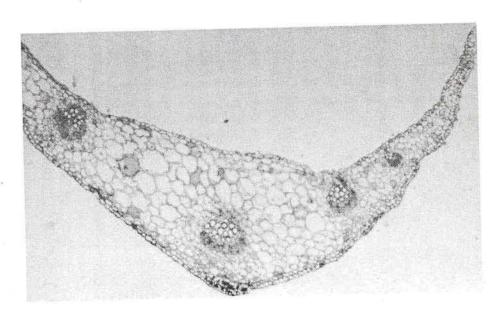


Fig.(5): Transverse section through the sheath of the third leaf on the main stem of Pimpinella anisum L. at the age of 6 weeks. (X 52)

thickened and covered with a cuticle layer. Stomata and trichomes are present. Trichomes are similar to those found on the leaf blades. The angles, beneath the epidermis, consist mainly of sclerenchyma. The ground tissue consists mostly of relatively large parenchyma cells with small triangular intercellular spaces, except at the distance between the angles where there are 2-3 layers of chlorenchyma cells directly underlying the epidermis (Fig.4).

The vascular tissues are formed of five main collateral bundles which are arranged in crescent shape, and separated from one another by wide spaces of ground tisse. The largest bundle (the median one) is located on the abaxial side of the petiole, and oriented with the xylem directed towards the adaxial side. Secretory canals are embedded in the parenchymatous ground tissue, each opposite to the phloem of every vascular bundle.

3.1.3. Leaf sheath

The sheath of Anise leaf as appeared in the transverse section shown in Figure (5) is nearly crescent in shape, more thicker at the middle portion and tapers towards the two ends. The sheath is bounded by a uniseriate epidermis of nearly square-shaped cells which is covered with a cuticle layer especially at the abaxial surface. Stomata are present, but trichomes are rarely found.

The ground tissue, especially at the middle portion of the sheath, consists mainly of relatively large polygonal parenchyma cells with obvious triangular intercellular spaces. Sclerenchyma strands composed of two layers of cells are present beneath the epidermis of the abaxial side only at the median portion of the sheath opposite to the large bundle. There are five collateral vascular bundles, embedded in the ground tissue, different in size and arranged in crescent shape. Secretory canals are present in the ground tissue at the tapering ends of the sheath and opposite to the phloem of each bundle.

3.2. Structure of the floral buds

Anise plant bears white flowers in the form of compound umbels. The flower is small actinomorphic, bisexual, epigynous and pentamerous except for the dimerous gynoecium. The sepals are absent. The petals are five, white in colour, distinct, with retuse apex, inflexed at the tip and valvate. The stamens are five, alternate with the

petals and borne on the nectary disc. Filaments are relatively long and inflexed. Anthers are tetrasporangiate, dithecal and opening lengthwise. Gynoecium is comprised of two united carpels. The ovary is inferior, bilocular, and in each loculus a single pendulous ovule hangs from the median septum, placentation is apical. Style two, thickened at the base together with a swollen nectary disc, yellow in colour and capping the ovary. Each style terminates in a spherical stigma. Ovary prominently ribbed and covered with numerous short trichomes.

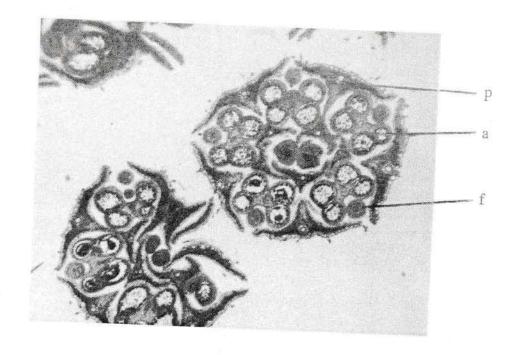
The transverse sections of the floral buds of Anise plant as seen in Figure (6) coincide to the above mentioned characteristics of Anise flower. It is evident that calyx is absent. Corolla, in transverse section, consists of five incurved petals. At the centre of each petal, there is one small vascular bundle embedded in the ground tissue and an obvious secretory canal opposite to the phloem of such bundle. The epidermal cells of the petal develop trichomes.

The stamens are five, alternate with the petals. The stamen consists of a two-lobed four-loculed anther borne on the filament, a thin stalk with a single vascular bundle. The filament is relatively simple in structure, parenchyma surrounds the vascular bundle and the epidermis may have trichomes. The vascular bundle traverses the entire filament and ends blindly in the connectissue located between the two anther halves.

Gynoecium is comprised of two united carpels. The ovary is bilocular, and in each loculus a single anatropous pendulous ovule hangs from the median septum, placentation is apical. Ovary prominently ridged, with obvious secretory canals (vittae), and covered with numerous short trichomes. Each carpel usually has five ridges; i.e., pentagonal in outline.

3.3. Structure of the fruit and the seed

Fruit of Anise is a dry schizocarp (cremocarp), ovate or broader than long, laterally compressed. The cremocarp consists of two mericarps each corresponding to one carpel containing one seed. Anise fruit does not split easily at maturity between carples and the two mericarps occur usually entire, with short length of the pedicel attached.



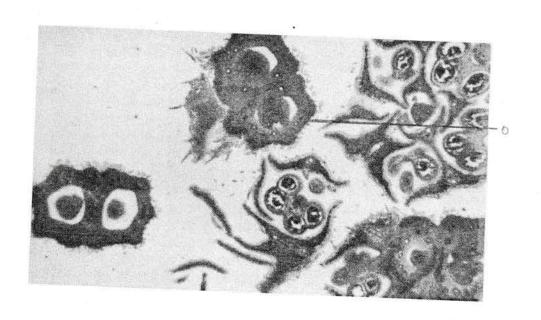


Fig.(6): Transverse sections of floral buds of Pimpinella anisum L.

Details: a, anther; f, filament; o, ovary and p, petal.

(X 52)

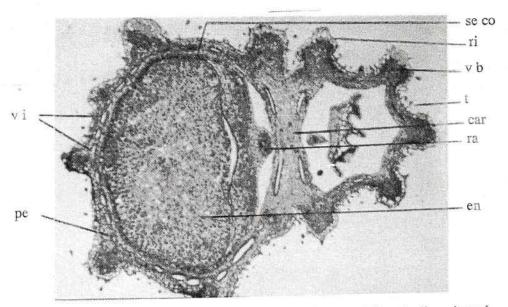


Fig.(7): Transverse section of the mature fruit (cremocarp) of Pimpinella anisum L., showing the structure of the fruit and the seed. (X 52)

Details: car, carpophore; en, endosperm; pe, pericarp; ra, raphe; ri, ridge; se co, seed coat; t, trichomes; v b, vascular bundle and vi, vittae.

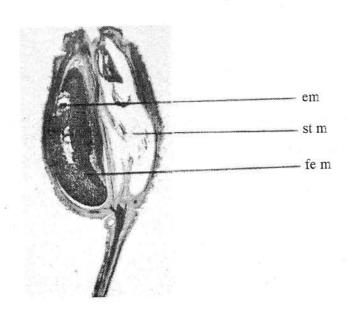


Fig.(8): Longitudinal section of the mature cremocarp of Pimpinella anisum L.
(X 24)

Details : em, embryo; fe m, fertile mericarp and st m, sterile mericarp.

Transverse and longitudinal sections of the mature fruit of Anise are shown in Figures (7) and (8); respectively. It is obvious that the fruit consists of two mericarps of which one is fertile (develops one fertile seed)and the other mericarp is usually sterile . Each mericarp has a flat surface (the commissural surface) and a rounded surface (the dorsal surface). From the central line of each commissural surface a fine thread separates, being attached basally to the pedicel and apically to the upper end of the mericarp. This thread is known as the carpophore. Each mericarp has two, sometimes three, large vittae on the commissural surface and about twenty to thirty small vittae on the dorsal surface. Between the vittae the pericarp is ridged externally and a vascular strand is contained in each of these ridges. The fertile mericarp usually has seven ridges, whereas the sterile one only has five ridges. The epidermis is characterized by numerous short, stiff trichomes. The ovule is anatropous and consequently on the commissural surface of the seed, a fine vascular strand, the raphe, extends from base to apex in the central line of the testa, which is wider in that region than elsewhere.

Anise fruit is orthospermous; *i.e.*, seed is flat on the inner surface. The seed is attached by its testa to the pericarp (schizocarpic fruit) so that it completely fills the loculus. The seed contains a small dicotyledonous embryo at the apical end, embedded in an abundant oily endosperm.

The previously mentioned structure of Anise fruit and seed is in harmony with those given by Fitting et al.,(1930), Parry (1945) and Wallis (1967).

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دراسات مورفولوجية وتشريحية على نبات الينسون من الفصيلة الخيمية ٤- التركيب التشريحي للأوراق والبراعم الزهرية والثمار

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ملخص

تم دراسة التركيب التشريحي للورقة والبراعم الزهرية والثمار الناضجة لنبات الينسون. جاء نصل الورقة الثالثة والورقة الثامنة على الساق الرئيسية وكذلك أنصال الأوراق على الأفرع الجانبية متماثل التركيب ،الورقة ذات جانبين ويعتبر الترتيب المنضغط لخلايا البشرة ووجود طبقة الأدمة والثغور والشعيرات من الملامح الرئيسية لبشرة ورقة نبات الينسون . تكون البشرة العليا والسفلي في منطقة العرق الوسطى مقعرة ومغطاة بطبقة أدمة سميكة خاصة على السطح السفلي . توجد الثغور والشعيرات على كلا سطحي الورقة ، والشعيرات إما وحيدة الخلية أو ثنائية الخلية أو مكونة من صف من ثلث خلايا . يتكون النسيج العمادي من طبقتين من الخلايا تشغل نصف من شلك النسيج الوسطى الميزوفيل) . تكون الحزم الوعائية في العروق الرئيسية مصحوبة مصن أعلى ومن أسفل بنسيج بارنشيمي مطمورا " به قناة إفرازية صغيرة أعلى الحزمة وقناة

إفرازية كبيرة أسفل الحزمة، وأحيانا تكون الحزمة محاطة بغلف من طبقة واحدة من خلايا بارنشيمية.

يكون عنق الورقة غالبا خماسى الأضلاع محاط ببشرة من طبقة واحدة من الخلايا مربعة الشكل تقريبا ويحتوى على ثغور وشعيرات . تتكون الزوايا ، اسفل البشرة ، أساسا من اسكلرنشيمة ، ويتكون النسيج الأساسي غالبا من خلايا بارنشيمية كبيرة نسبيا فيما عدا المنطقة التي بين الزوايا حيث تتكون من ٢ __ ٣ طبقات من خلايا كلورنشيمية أسفل البشرة مباشرة . تتكون الأنسجة الوعائية من ٥ حزم جانبية رئيسية مرتبة بشكل هلالي يفصلها عن بعضها البعض مسافات واسعة من النسيج الاساسي . توجد القنوات الإفرازية مطمورة في بارنشيمية النسيج الأساسي وتكون مواجهة للحاء كل حزمة وعائية.

يحاط غمد الورقة هلالى الشكل تقريباً ببشرة من طبقة واحدة من الخلايا بها ثغور ونادراً ما توجد بها شعيرات. يوجد ٥ حزم وعائية جانبية مطمورة فى النسيج الأساسى مختلفة فى الحجم ومرتبة بشكل هلالى. توجد القنوات الإفرازية فى النسيج الأساسى عند طرفى الغمد وكذلك مقابل لحاء كل حزمة وعائية.

وجد بالنسبة للبراعم الزهرية ، أن سبلات الكأس غائبة والتويج يتكون من مبتلات منحنية وتحتوى خلايا البشرة على شعيرات ، ويوجد في مركز كل بتلة حزمة وعائية صغيرة مطمورة في النسيج الأساسي كذلك توجد قناة إفرازية مقابل لحاء هذه الحزمة . يتكون الطلع من ٥ أسدية منفصلة متبادلة مع البتلات ، وتتكون السداة من متك ذو فصين و ٤ حجرات ويحمل على خيط رفيع به حزمة وعائيسة واحدة . ويتكون المتاع من كربلتين ملتحمتين ، المبيض ذو مسكنين ويوجد فسي كل مسكن بويضة منعكسة مدلاه ومعلقة من الحاجز الوسطى والوضع بالمشيمي قمي . أما المبيض فهو ذو أضلاع بارزة وبه قنوات إفرازية ومغطى بالعديد مسن الشعيرات القصيرة ، كل كربلة بها ٥ أضلاع عادة.

تتركب الثمرة من ثميرتين أحدهما خصبة (ينتج عنها بنرة خصبة) والثميرة الأخرى عقيمة غالبا . كل ثميرة بها قناتين وأحيانا ثلاث قنوات زيتية كبيرة على السطح الداخلى (البطني) وحوالى ٢٠ ــــ ٣٠ قناة زيتية صغيرة على السطح الخارجي (الظهرى) . يكون السطح الخارجي للغلاف الثمرى مضلع بين القنوات الزيتية ويوجد بكل ضلع حزمة وعائية . تتصل البذرة من خيلال القصرة بالغلاف الثمرى (ثمرة خيمية منشقة) وعلى ذلك تملأ البذرة المسكن . تحتوى البذرة على جنين صغير ذو فلقتين يوجد في النهاية الطرفية للبذرة (القمية) ومطمورا "في كمية كافية من الإندوسبرم الزيتي.

المجاة العلمية لكلية الزراعة _ جامعة القاهرة _ المجلد (٥٢) العدد الرابع (أكتوبر ٢٠٠١): ٥٠ - ٧٧٠.