

FOLIAR ANATOMICAL STUDIES IN THE UMBELLIFERAE FROM ARASBARAN, IRAN

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The foliar anatomy of the *Umbelliferae* family from Arasbaran Protected Area including following species were studied under light microscope: *Albovia tripartita*, *Bupleurum falcatum*, *Bupleurum gerardii*, *Carum carvi*, *Caucalis platycarpus*, *Cervaria caucasica*, *Chaerophyllum aureum*, *Cymbocarpum anethoides*, *Daucus broteri*, *Eryngium caasicum*, *Falcaria vulgaris*, *Heracleum pastinacifolium*, *Heracleum persicum*, *Laser trilobum*, *Pimpinella aurea*, *Pimpinella tragium*, *Prangos ferulacea*, *Sanicula europaea*, *Seseli peucedanoides*, *Trinia leiogona*. The different anatomical characters on *Umbelliferae* indicated flexibility of this family in various ecological circumstances.

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Key words. Foliar anatomy, *Umbelliferae*, stomata, trichomes, secretory ducts, mesophyll, Iran, protected area, environmental factors.

ساختمان تشریحی برگ چتریان ارسباران، ایران

فاطمه زرین‌کمر و عادل جلیلی

ساختمان تشریحی برگ گونه‌هایی از جنسهای مختلف تیره جعفری جمع‌آوری شده از منطقه حفاظت شده ارسباران مطالعه گردید. مشخصات برگ در سطح و در برش عرضی بیانگر صفات تشریحی جالبی می‌باشد. بر اساس همین صفات تاثیرگذاری عوامل اقلیمی بر روی گونه‌ها بحث و بررسی شد.

INTRODUCTION

The *Umbelliferae* is a large and widely distributed family and was probably the first to be recognized by taxonomists, because of its characteristic inflorescence and fruits. It contains 180 genera with about 3000 species dispersed throughout the world, especially in the North Hemisphere (Heywood 1971). From an economic and medicinal point of view the family is of great interest. Certain species of the *Umbelliferae* are eaten as vegetable, others are the source of gum resins used in perfumery and medicine, and a third group yields other products of medicinal value (Metcalf, 1960). Some species of the family are well known either because they have medicinal properties, or because of the great amount of essential oils they contain. Serious cases of poisoning have been caused by a few species. Some of them, such as hemlock (*Conium maculatum*), exemplify very strong poisons. All *Umbellifers* are aromatic plants. They produce essential oils and biogenetically related resins which are excreted in schizogenous canals in roots, stems, leaves, inflorescence and fruits (Heywood 1971 and Hegnauer 1971). The family is well represented in the flora of Iran by about 120 genera, 350 species and 100 endemic plants (Mozaffarian 1996). The plants are annual or perennial herbs, rarely shrubby as *Bupleurum*, with a wide range in habitat, namely stout erect stems with hollow internodes, alternate pinnately exstipulate sheathing leaves and compound generally white flowers in umbels (Minosuke 1958). This research aims to provide useful information on some 20 species of the *Umbelliferae*

MATERIALS AND METHODS

The species under discussion in this paper were collected from Arasbaran Protected Area in northwest of Iran. Materials were fixed in FAA and transverse sections of leaf were

prepared by hand cutting. Sections were cleared with sodium hypochlorite, dehydrated and colored with methyl green and carmine-vest and mounted in gelatin. To minimize misinterpretation, the central area of the leaf lamina from relatively mature leaves was selected for analysis, and at each site, the data entry comprises an average of 30 samples taken from 6 plants of the same species. In order to study stomata density, the diafanization technique was employed (Stritmatter, 1973), and the results were observed using a light microscope.

The species under study in this paper and locality of them are as follows:

Albovia tripartita Schischk. -Arasbaran, Kalale Olia (research forest), 1400 m, NE, 60%, Hamze'ee & Asri.

Bupleurum falcatum L. -Arasbaran, Between Mahmoodabad & Makidi, 2120 m, S, 60-70%, Hamze'ee & Asri.

Bupleurum gerardii All. - Arasbaran, Between Makidi & Shojaabad, 450 m, NW, 55% Hamze'ee & Asri, 81787, TARI

Carum carvi L. - Arasbaran, Between Abbasabad & Mahmoodabad highland, 2150 m, NW, 50%, Hamze'ee & Asri.

Caucalis platycarpos L.- Arasbaran, Armany Oulan near to Vayghan, 1400 m, SE, 45%, Hmze'ee & Asri.

Cervaria caucasica (M. B.) Pimenov. - Arasbaran, low elevations of Toopkhaneh highland 1600 m, NW, 55%, Hamze'ee & Asri.

Chaerophyllum aureum L.- Arasbaran, Abbasabad highland, 2426 m, N, 60%, Hamze'ee & Asri.

Cymbocarpum anethoides DC.- Arasbaran, Between Asheghloo & Kalale, 358 m, SW, 60%, Hamze'ee & Asri.

Daucus broteri Ten. - Arasbaran, Tazehkand road, 1300 m, N, Hamze'ee & Asri. 81975, TARI

Eryngium caucasicum Trautv. - Arasbaran, Between Mahmoodabad & Makidi, 1800 m, SE, 50%, Hamze'ee & Asri. 81791, TARI

Falcaria vulgaris Bernh. - Arasbaran, Makidi near Kalibar, 1450 m, 0, Hamze'ee & Asri.

Heracleum pastinacifolium C. Koch - Arasbaran, Abbasabad highland, 2426 m, N, 60%, Hamze'ee & Asri.

Heracleum persicum Desf. ex Fischer - Arasbaran, Abbasabad highland, 2426 m, N, 60%, Hamze'ee & Asri.

Laser trilobum (L.) Borkh. - Arasbaran, Kalale Olia (research forest), 1400 m, NE, 60%, Hamze'ee & Asri.

Pimpinella aurea DC.- Arasbaran, Toopkhaneh highland, 2350 m, NW, 45%, Hamze'ee & Asri.

Pimpinella tragiium Vill. - Arasbaran, Abbasabad highland, 2426 m, N, 60%, Hamze'ee & Asri.

Prangos ferulacea (L.) Lindl. - Arasbaran, Abbasabad highland, 2426 m, N, 60%, Hamze'ee & Asri.

Sanicula europaea L. - Arasbaran, Low elevations of Toopkhaneh highland, 1600 m, NW, 55%, Hamze'ee & Asri.

Seseli peucedanoides (M. B.) Kos.- Pol. - Arasbaran, Kalale Olia (research forest), 1420 m, NW, 50%, Hamze'ee & Asri.

Trinia leiogona Hoffm. - Arasbaran, Between Makidi & Shojaabad, 450 m, N Hamze'ee & Asri.

OBSERVATIONS

Superficial view

Epidermis; In general, it consists of cells with sinuous anticlinal walls, on abaxial surfaces except in *Bupleurum gerardii* and *Pimpinella tragiium*, which is smooth on adaxial surfaces in all species (Fig.2, D, E). The epidermis is provided with a thick **cuticle**, in certain species becomes striate shape on both surfaces, frequently with large papillous over the veins. **Stomata**, with the exception of some species are present on both surfaces. Stomata frequencies with the exception of the genus *Bupleurum* on abaxial surfaces are greater than on adaxial surfaces. The highest frequency is observed in abaxial surface of *Pimpinella aurea* with the average of 533 per mm²(Table 1). In the *Prangos ferulacea* stomata frequency is similar on both surfaces. The density differences between both surfaces show variability between species. Various species of this family are found in very different habitats and the types and sizes of stomata are variable, even intra-specifically. For example in *Chaerophyllum aureum*, the commonest stomata type is the anisocytic pattern at (40%), followed by anomocytic (30%), then diacytic (20%) and last frequently paracytic (10%). Because of environmental variation, taxonomic classification is particularly difficult. In the *Umbelliferae*, stomatal types provide a valuable indicator for easier identification and classification at the species level. The average of guard cell length is between 17.66-45.3µM both of them on abaxial surface. As the Table 1, indicates, the types of stomata are highly variable in herbaceous species, and so could be a valuable tool to inform taxonomic classifications (Fig.1, 2, A-H). **Trichomes;** in the considerable species leaf surface is glabrous. However when trichome found on both surfaces adaxial densities are higher than those of abaxial surfaces throughout the rest of

Table 1. Anatomical characters of *Umbelliferae* family from Arasbaran, Iran. (dia=diacytic; anomo=anomocytic; aniso=anisocytic).

Species	Density of stom. adx (mm ²)	Length of stom. adx (µm)	Length of stom. abx (µm)	Type of stomata	Density of th. ch. adx (mm ²)	Density of th. ch. abx (mm ²)	Type of trichome	Cuticle (µm)		Type of mesophyll	Crystal
								adaxial	abaxial		
<i>Albivia tripartita</i>	3.7	144.8	25.32	dia>anomo	<10%	numerous	simple	5	2	dorsiventral	-
<i>Euplaeum falcatum</i>	210	126.67	17.7	aniso>anomo	glabrous	glabrous	-	5	8	dorsiventral	-
<i>Euplaeum gerardii</i>	183.46	165.56	17.66	aniso	glabrous	glabrous	-	5	7	dorsiventral	-
<i>Caram carvi</i>	117.5	289.2	27.367	aniso>anomo	glabrous	glabrous	-	4	4	dorsiventral	-
<i>Caucalis platycarpus</i>	91.8	225.7	27.83	dia	<10%	<10%	simple	4	3	dorsiventral	-
<i>Cervaria caucasia</i>	-	173.4	22.76	anomo	glabrous	glabrous	-	5	2	dorsiventral	-
<i>Chaerophyllum aureum</i>	4.44	326.7	21.76	anomo=aniso	<10%	<10%	simple	6	4.5	dorsiventral	-
<i>Cymbocarpum anethoides</i>	111.81	107.59	30.73	anomo	glabrous	glabrous	-	3	4	isobilateral	-
<i>Daucus broteri</i>	216.7	300	25.2	dia	glabrous	numerous	simple	2.5	4	isobilateral	-
<i>Eryngium caucasicum</i>	91.61	107.53	26.16	aniso	glabrous	glabrous	-	15	15	isobilateral	druse
<i>Falcaria vulgaris</i>	88.06	107.9	22.66	dia	glabrous	glabrous	-	8	10	dorsiventral	-
<i>Heraclium pastinacifolium</i>	122.85	298.47	28.33	anomo>aniso	<10%	>10%	simple	8	8	dorsiventral	-
<i>Heraclium persicum</i>	117.14	275.5	24.61	anomo>aniso	glabrous	numerous	simple	4	2	dorsiventral	-
<i>Laser trilobum</i>	-	108.87	29.62	anomo	glabrous	glabrous	-	3.6	2.4	dorsiventral	-
<i>Pimpinella aurea</i>	105	533.3	21.6	aniso>anomo	numerous	numerous	simple	5	5	isobilateral	-
<i>Pimpinella tragiun</i>	101.32	157.5	26.531	dia, aniso	numerous	numerous	simple	10	9	isobilateral	-
<i>Prangos ferulacea</i>	50	38.1	45.3	aniso>dia	glabrous	glabrous	-	10	7	centric	raphid
<i>Santalia europaea</i>	-	90.35	22.96	anomo	glabrous	glabrous	-	4.8	5	dorsiventral	prisma
<i>Seseli peucedanoides</i>	-	222.86	28.7	aniso>anomo	glabrous	glabrous	-	4	6	dorsiventral	-
<i>Thymus latogona</i>	93.88	87.55	26.66	anomo>aniso	glabrous	glabrous	-	5	5	isobilateral	-

family. The trichome is simple, short and rigid, present over the veins and leaf margin on adaxial surface and in medium length on abaxial, including unicellular and multicellular (up to 3 cells). Sometimes incrustated with small crystals (Fig.5, C-F; Fig.6, A).

Transversal section

Epidermis, Uniseriate (single layer), it consists the large tetragonal cells with thick outer walls. In general, adaxial epidermal cells two times larger than abaxial (Fig.3, E, F). Stomata subsidiary cells are small respect to other epidermal cells. **Papilla** upper midrib and leaf margin (Fig.5, B; Fig.8, C, D, F). **Cuticle**, striated, about 2-15 μ . **Stomata** are usually superficial on both surfaces, (Fig.4, A-D; Fig.5, C-F; Fig.7, D-G) with the exceptions of *Bupleurum species*, *Eryngium caucasicum* and *Pimpinella aurea* where stomata are completely sunken (Fig.8, E). **Hypoderm**, form a continuous layer below the abaxial surfaces in *Eryngium caucasicum*, *Pimpinella species* and *Prangos ferulacea* (Fig.4, F; Fig.8, B). **Mesophyll** often is dorsiventral including one layer of palisade cells (Fig.3, A-F; Fig.6, A-C). Spongy mesophyll provided large intercellular spaces and tending to be in variously branched in *Sanicula europaea* and *Caucalis platycarpus* (Fig.6, F-H; Fig.7, A). Isobilateral mesophyll occasionally recorded in certain species with two dense palisade layers in each side (Table 1). It is also observed that in this type bundle sheaths extend into mesophyll and form 2-3 layers of isodiametric parenchymatous cells in the middle of mesophyll (Fig.5, A-E; Fig.6, D). Mesophyll is centric in *Prangos ferulacea* (Fig.4, F).

Vascular bundels are collateral, surrounded by parenchymatous bundle sheaths. In midrib and some large veins, bundle sheaths extend toward abaxial or both epidermis, by angular collenchyma or large packet of sclerenchyma. Interestingly, that also occur at the leaf margin in *Eryngium caucasicum* (Fig.8, A, C, D). Midrib has an adaxial appendix translucent by collenchyma in *Sanicula europaea* (Fig.7, B). This study shows that all of the vascular bundles have a secretory ducts, in both pole of veins or in periphloematic zone, which create in schizogenous canal with an epithelium that surrounds a central cavity and yellow contents (Fig.6, G; Fig.8, D). **Crystals**, numerous crystals in different type (druses, raphid, and prisma) present in epidermal cells and also accompanying the vascular bundles of the vein (Fig.7, C).

Secretory ducts

Ducts are elongated cavities. They can often branch to create a network extending from the roots through the stem to the leaves, flowers and fruits. They are composed of an epithelium that surrounds a central cavity. Some cells within the parenchyma undergo asynchronous division and in doing so, they expand the initial space in the middle, where the cells are adjacent, to form a cavity. Some of these cells forming the wall of the cavity will change into secretory epithelial cells, which are excreted in schizogenous canals in roots, stems, leaves, The oils are biosynthesized within their leucoplasts and move via the endoplasmic reticulum into the cavity. These cavities then become joined to form ducts. They can be found in all of the **Umbelliferae** family. The

essential oils contain mainly monoterpenes, sesquiterpenes and phenyl-propanoid compounds. Many well-known essential oil were isolated for the first time from an Umbelliferous plant and were named (Hegnauer, 1971).

Discussion

In foliar study of 20 species of the *Umbelliferae* in various genera, interesting anatomical characters were observed. One of the most notable characters in *Umbelliferae* family is its flexibility to adapt internal structure to environmental conditions, therefore, causes difficulties for taxonomists for easily recognition of species in this family. Several authors have drawn attention to the structural plasticity of certain members of the *Umbelliferae*. For example, it was pointed out that leaf morphology varies *vis-a-vis* plant environment, particularly with water availability as well as light intensity. Environmental factors influence shaping multiple levels of adaptation, including, morphological, anatomical, physiological, and biochemical responses of *Umbelliferae* species. It could be, therefore, obvious that a variety of mesophyll types might observe amongst these species. Table 1 shows all three types of mesophyll are present while interestingly such traditional division of mesophyll type can be fluctuated. It means the structural configurations of mesophyll are flexible and some times mixture of two or even three types. For example mesophyll are dorsiventral in *Sanicula europaea* and *Heracleum pastinacifolium* while in one palisade cells are short and spongy cells with a voluminous intercellular system and in the others palisade cells are elongated and with dense spongy. That means there is tendency to be isobilateral in some dorsiventral species. In others species mesophylls are isobilateral with

an exception of *Prangos ferulacea* which contains centric mesophyll (Fig.4, F). The flexibility and plasticity of mesophyll in some species is obvious. As Proseus (2000), believes the shape and arrangement of photosynthetic cells responses to environmental factors like temperature and water availability. Based on his study increasing in leaves tissue density under water limitation are due to anatomical changes in the leaves, specifically, decreases in Photosynthetic cells (Proseus 2000). Measuring palisade layer and maximum hydraulic conductivity will test this hypothesis. The present study makes clear that together with temperature and water availability, these arrangements are also influenced by altitude (see Table 1, and Materials and Methods).

Plant responses to specific environmental stresses such as drought and flooding, light intensity, high heat, chilling and freezing, (Erik, et al 1996). The function of surfactants in such condition is to reduce surface tension, improve leaf surface spreading and sticking, and improve herbicide absorption. In present study some characters like the existence of trichome on adaxial surface or hypoderm and thickness of cuticle varies according to environmental conditions. The cuticle is a complex waxy layer secreted by epidermal cells. It forms a barrier to water loss and the entrance of pathogens. As it is obvious in *Eryngium caucasicum* and *Heracleum pastinacifolium* cuticular barrier causes restriction of effect of environmental conditions on plant factors (Fig.7, 8,E). According to Wagner (1999) and Wilson (2000). The correlation between the epidermal cells density and its measurement is response to environmental changes. That meant to be used as drought indicators. The epidermal cell size also is important because recent research makes clear that correlation have been found between water availability and temperature on the one side and the epidermal cell size on the

other side. Water deficit causes smaller epidermal cells (Ristic, 1991), and low temperatures diminish cell growth (Proseus, 2000). As it shows in *Seseli peucedanoides* variation in size and structure of epidermal cells in different parts of the leaf's upper and lower sides specially are the consequence of direct contact with the external environment (figure. 3, E, F). It evidences many important adaptations, which allow plants to survive.

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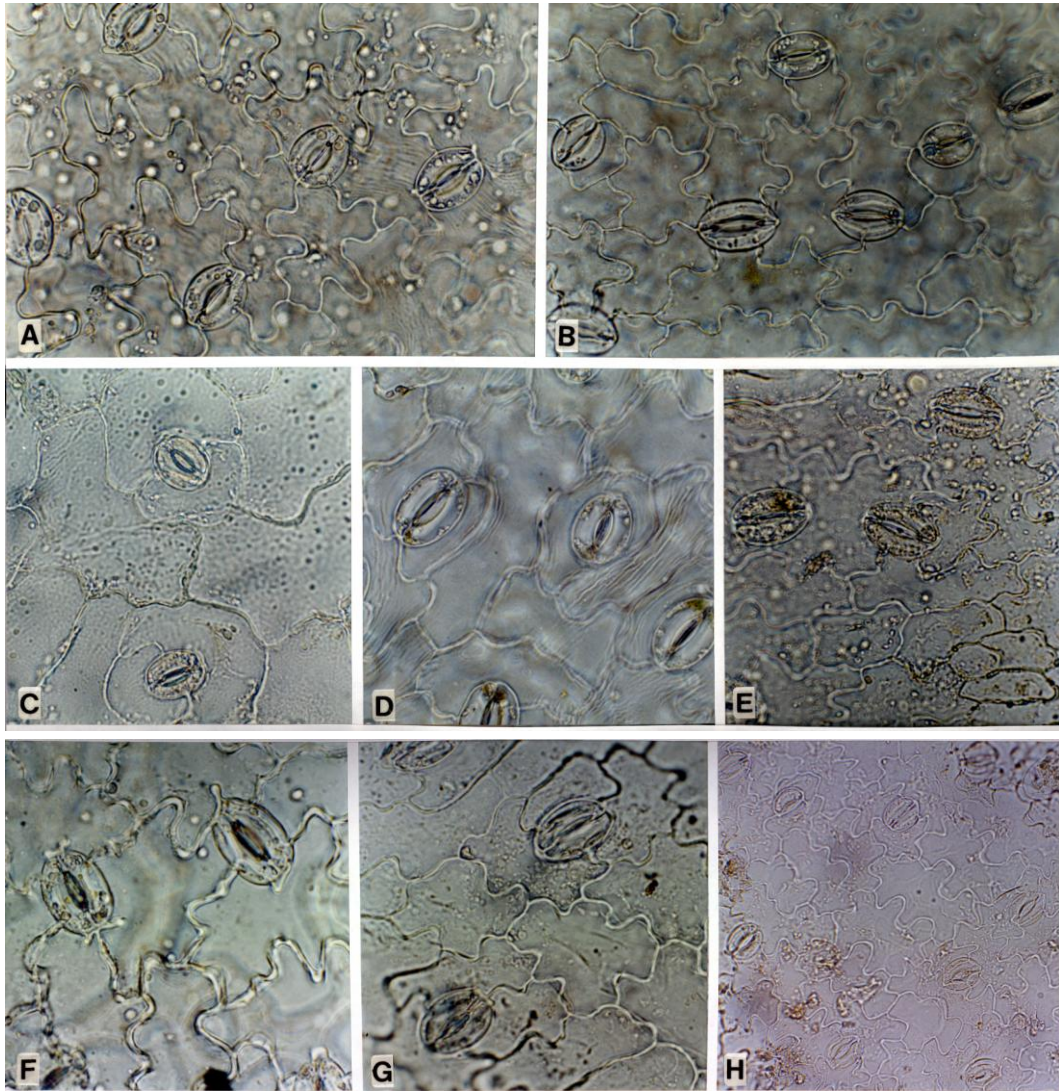


Figure 1: A-H, epidermis in superficial view of the *Umbelliferae*; A-E, H, abaxial; F, G, adaxial; A, *Cerevaria caucasica*; B, *Chaerophyllum aureum*; C, *Falcaria vulgaris*; D, *Seseli peucedanoides*; E, *Heracleum persicum*; F, *Sanicula europaea*; G, H, *Trinia leiogona*; A-G, ($\times 300$); H, ($\times 150$).

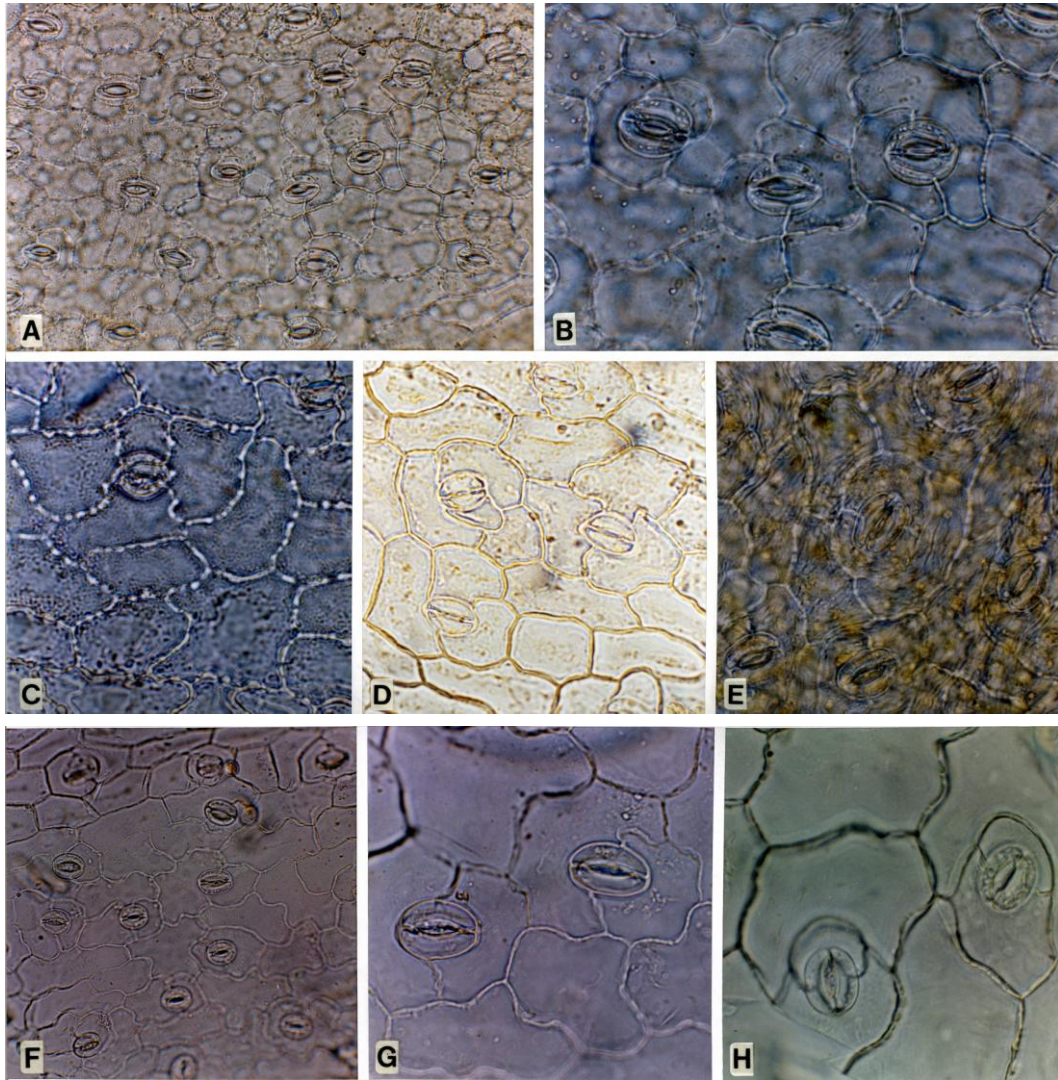


Figure 2: A-H, epidermis in superficial view of the *Umbelliferae*; A-E, G, abaxial; F, H, adaxial; A, B, *Carum carvi*; C, *Bupleurum falcatum*; D, *B. gerardii*; E, *Pimpinella tragiium*; F, G, *Daucus broteri*; H, *Caulalis platycarpus*; A, F, ($\times 150$); B-E, G, H, ($\times 300$).

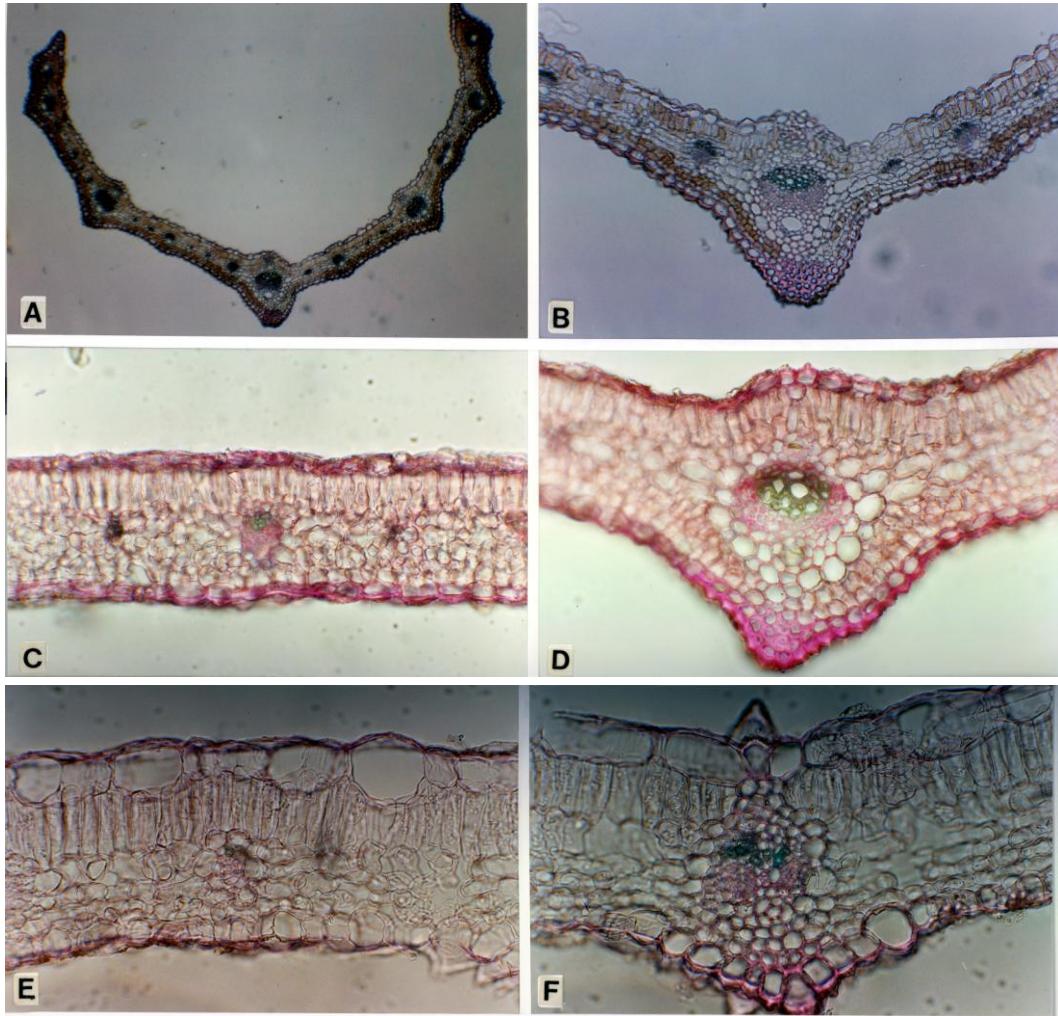


Figure 3: A-F, leaf of the *Umbelliferae* in Ts; A, B, *Bupleurum falcatum*; A, general aspect; B, central vein; C, D, *Bupleurum gerardii*; C, detail of mesophyll; D, central vein; E, F, *Seseli peucedanoides*; E, detail of mesophyll; F, central vein; A, ($\times 30$); B, ($\times 77$); C-F, ($\times 150$).

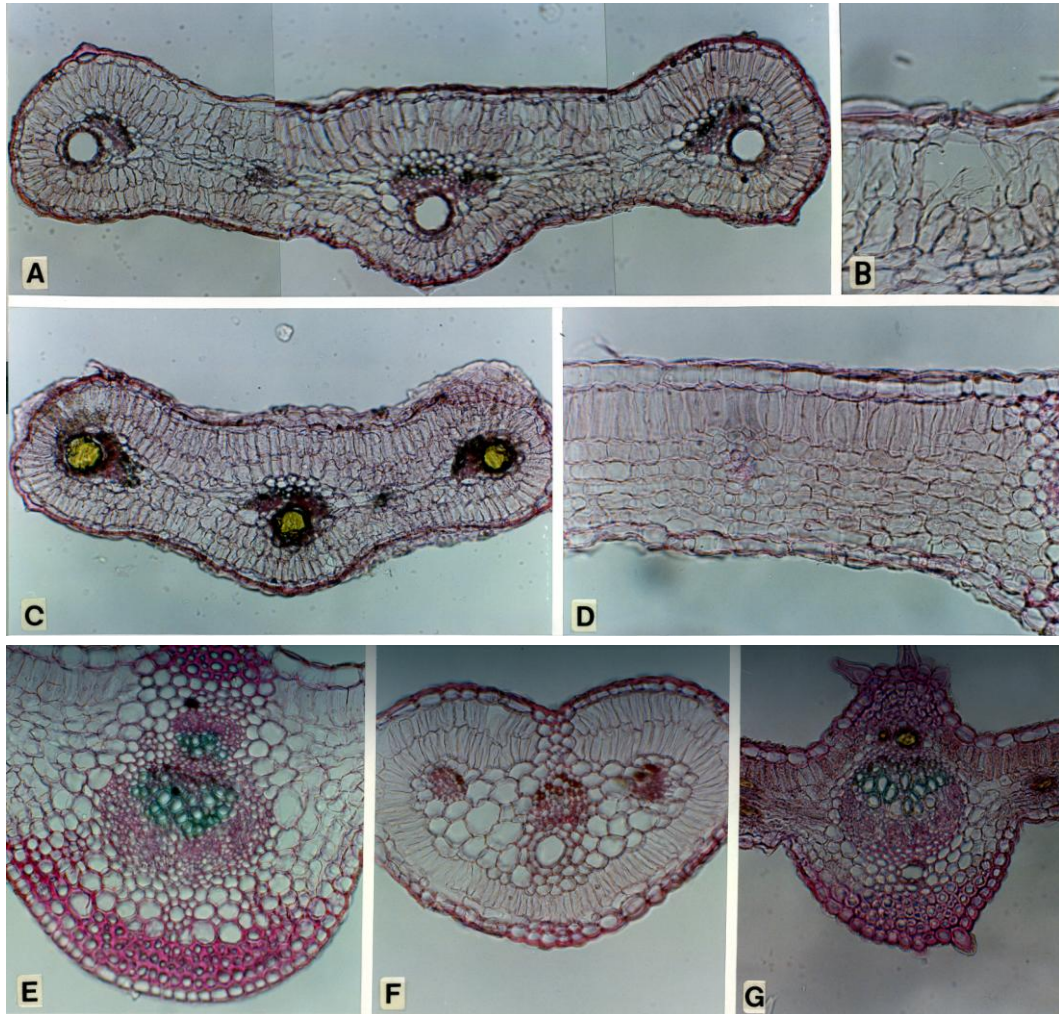


Figure 4: A-G, leaf of the *Umbelliferae* in Ts; A-C, *Trinia leiogona*; A, general aspect; B, stoma on the upper side; C, general aspect with Secretory canals containing mixture of oils, resin and mucilage; D, E, *Laser trilobum*; D, detail of mesophyll; E, central vein; F, general aspect of *Prangos ferulacea*; G, central vein of; *Cerevaria caucasica* A, C, E-G, ($\times 77$); B, D, ($\times 150$).

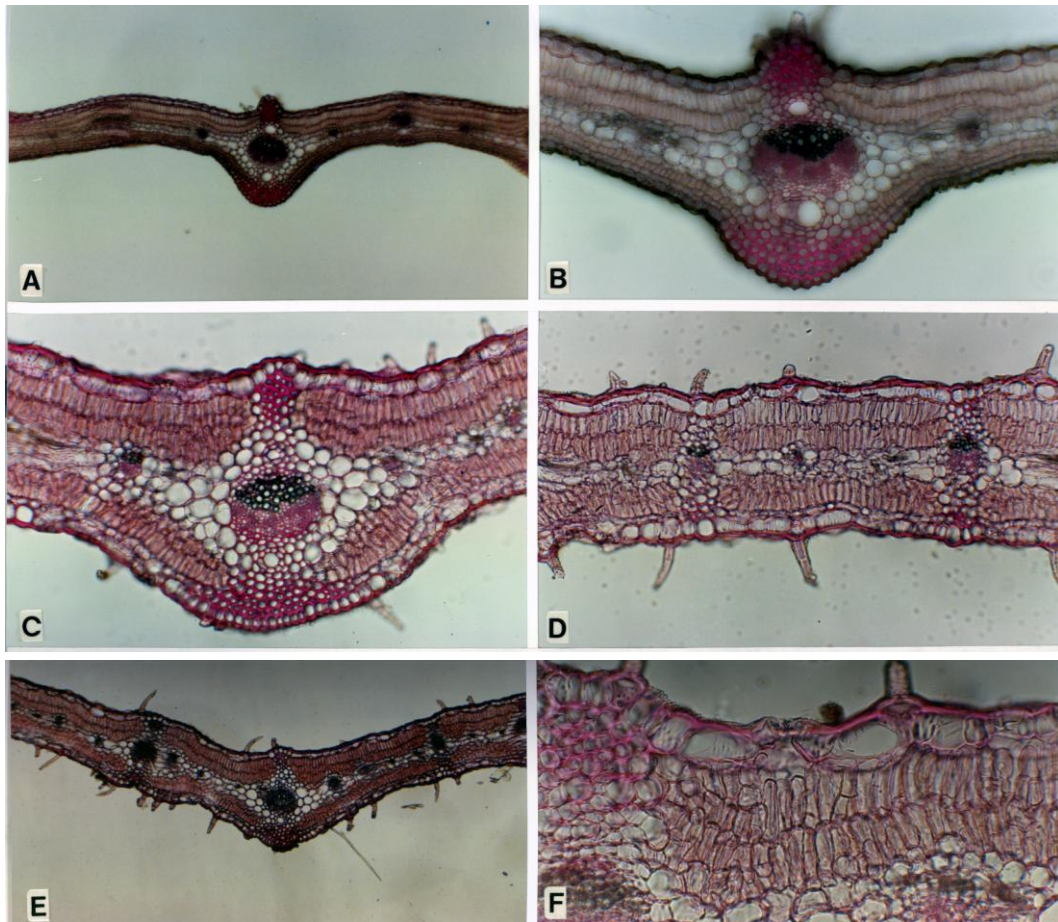


Figure 5: A-F, leaf of *Pimpinella* spp. in Ts; A, B, *P. aurea*; A, general aspect; B, central vein; C-F, *P. tragiium*; C, central vein; D, detail of mesophyll; E, general aspect; F, stoma on the upper side; A, E, ($\times 30$) B-D, ($\times 77$); F, ($\times 150$).

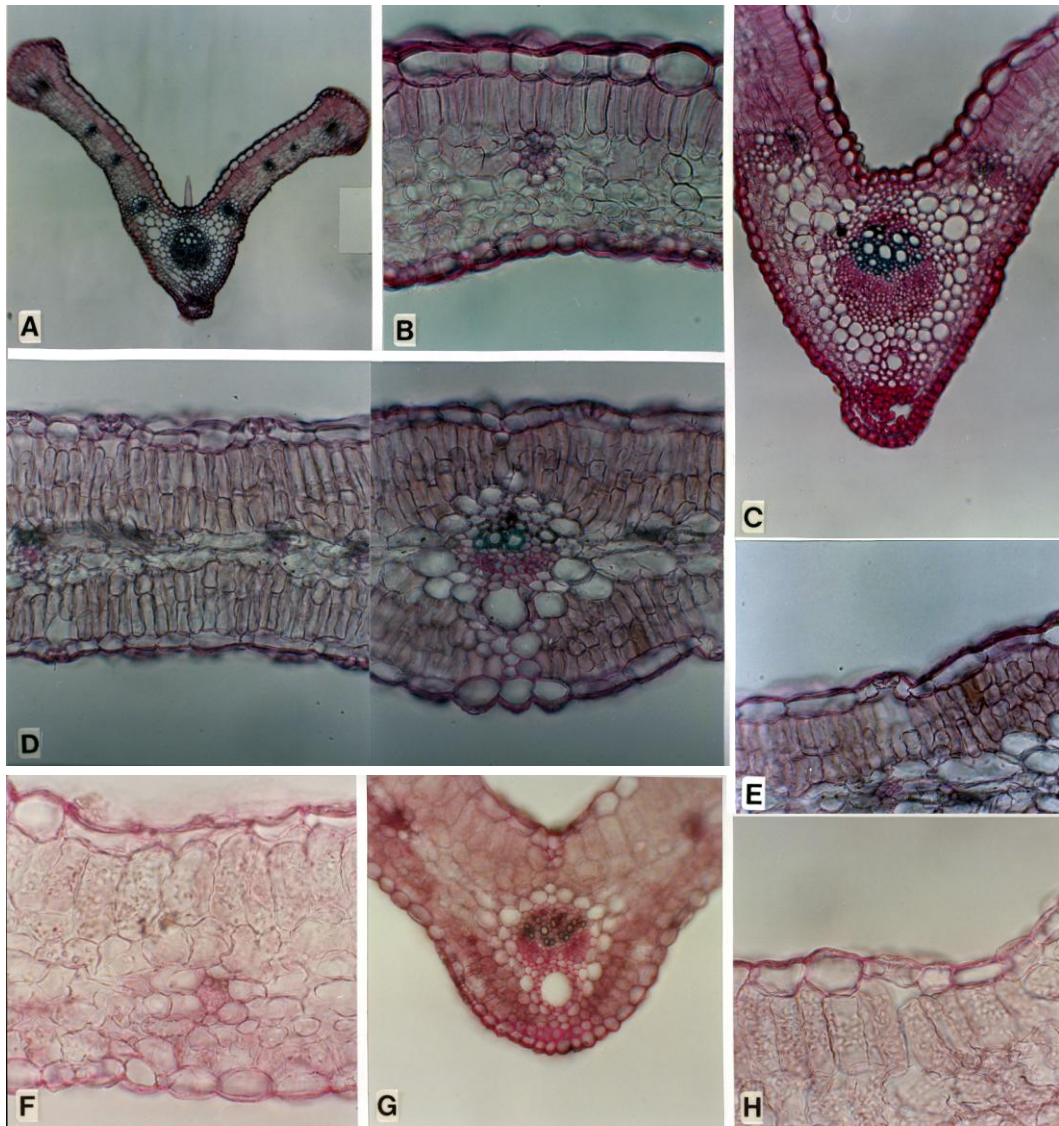


Figure 6: A-H, leaf of the *Umbelliferae* in Ts; A-C, *Chaerophyllum aureum*; A, general aspect; B, detail of mesophyll; C, central vein; D, E, *Daucus broteri*; D, general aspect; E, stoma on the lower side; F-H, *Caucalis platycarpos*; F, detail of mesophyll; G, central vein; H, stoma on the upper side; A, ($\times 30$); B, D-F, H, ($\times 150$); C, G, ($\times 77$).

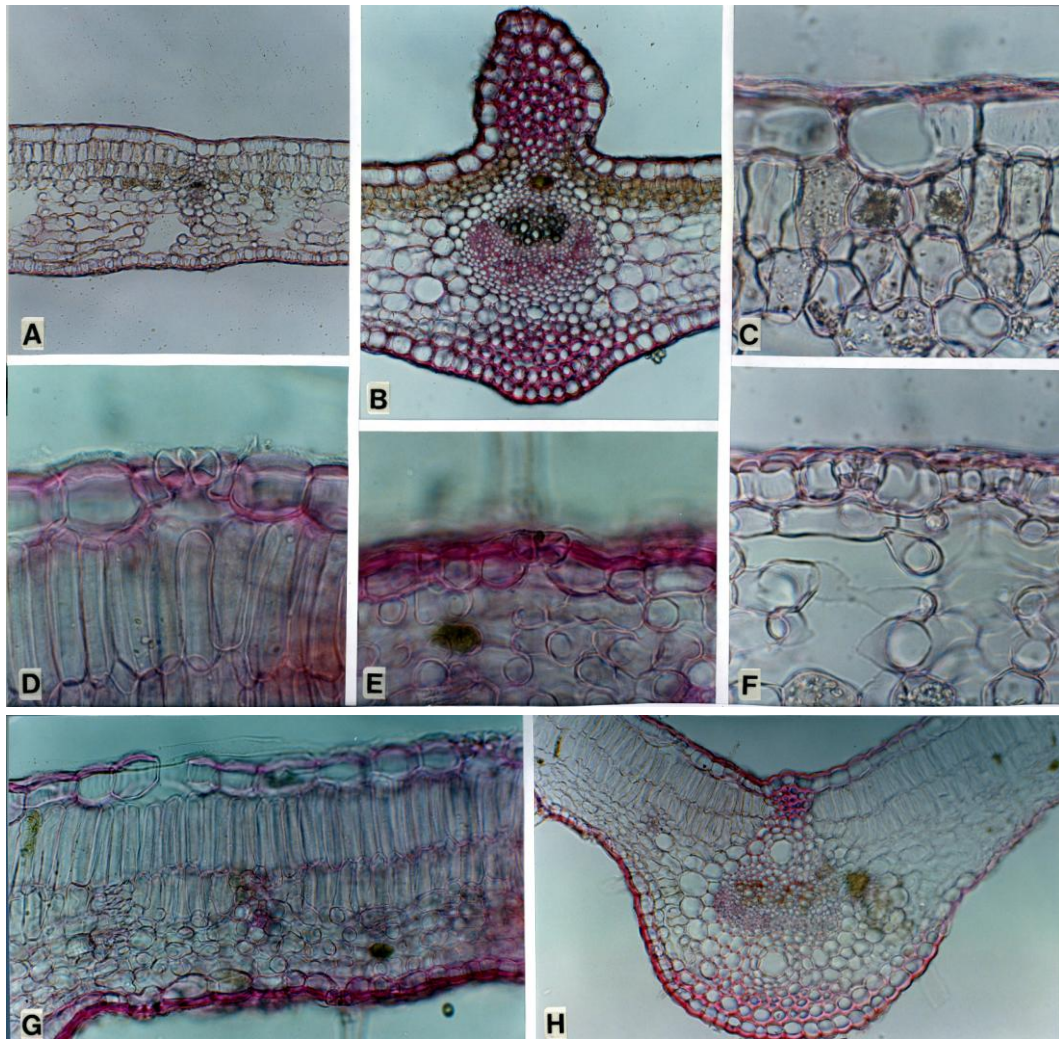


Figure 7: A-H, leaf of the *Umbelliferae* in Ts; A-C, F, *Sanicula europaea*; A, detail of mesophyll; B, central vein; C, crystal (druse) in the mesophyll; F, stoma on the lower side; D, E, G, H, *Heracleum pastinacifolium*; D, E, stomata; D, adaxial; E, abaxial; G, detail of mesophyll; H, central vein; A, B, H, ($\times 77$); C-F, ($\times 300$); G, ($\times 150$).

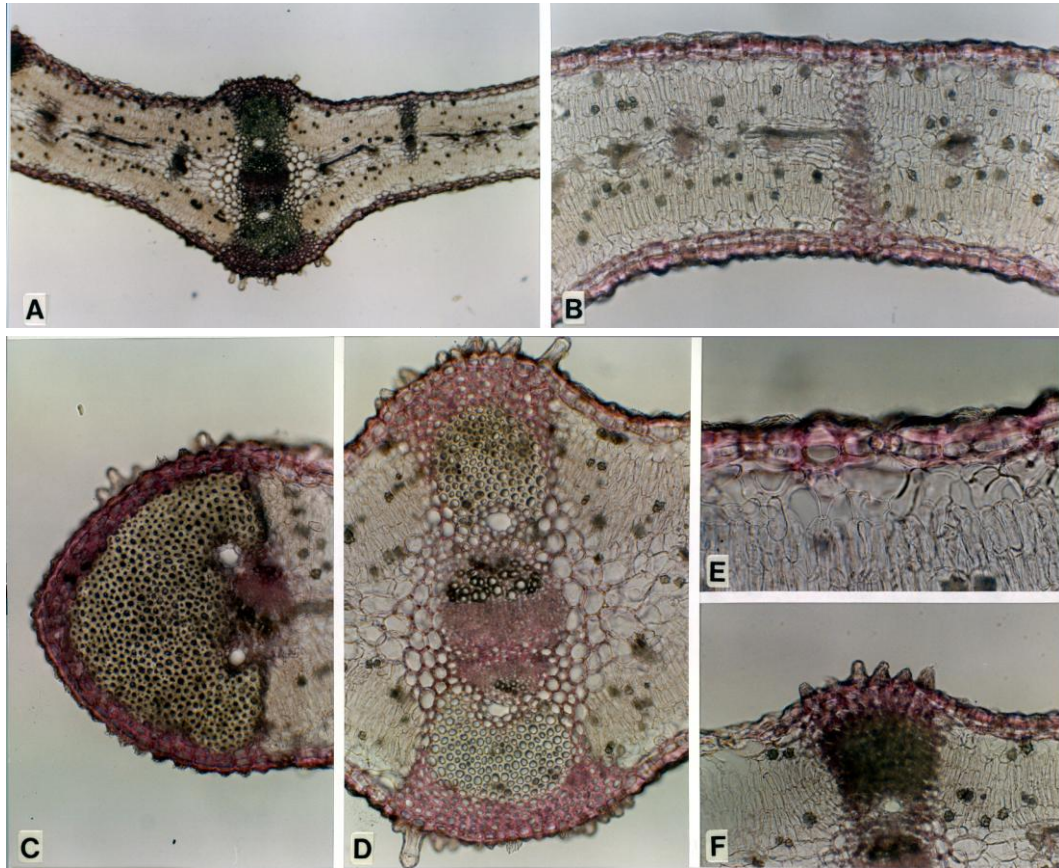


Figure 8: A-F, leaf of *Eryngium caucasicum* in Ts; A, general aspect; B, detail of mesophyll; C, collenchyma in the marginal region; D, central vein; E, stoma on the lower side; F, papillose epidermis particularly on the central vein; A, ($\times 30$); B-D, F, ($\times 77$); E, ($\times 150$).