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Nutlet anatomy of the genus *Salvia* L. in Jordan

Abstract

Oran, S. A.: Nutlet anatomy of the genus *Salvia* L. in Jordan. — Fl. Medit. 7: 27-40. 1997 — ISSN 1120-4052.

The nutlet anatomy of 19 Jordanian *Salvia* L. *(Labiatae)* was studied. Transverse sections were prepared using wax embedding and spurr medium techniques. Significant differences were observed between the species in coat thickness and mucilage production. Epicarp thickness was found to range from 5-75 µm, the mesocarp 13-75 µm thick and the endocarp 18-125 µm thick. Sclereids were the dominant feature of the endocarp; the following types were recognized: columnar (macrosclereids) with large or minute lumina, and osteosclereids with large and small lumina. The anatomical characteristics studied were found to be taxonomically useful and provided extra evidence for the separation and characterization of the species studied.

Introduction

Studies of the internal structure of seeds have played a significant role in plant taxonomy. A comprehensive study (Wojciechowsk 1958) has dealt with taxonomy, morphology and anatomy of fruits and seeds of the genus *Salvia* L. The anatomical criteria used included the structure of seed- coat cells and of the sclerenchymatic layer of the pericarp. The study showed that the differences in structure of the ribbed epidermal cell membrane of the seed coat in *Salvia viridis* var. *viridis* and *S. viridis* var. *horminum* are small but sufficiently distinct to maintain the two taxa.

A study of the anatomy of nutlets of *Salvia* from Afghanistan was carried out by Hedge (1970) to examine the production of mucilage. The study was supplemented by examination of transverse sections of the pericarp. His study showed that there are obvious differences in the thickening of the pericarp and in the properties of its individual layers.

Pericarp structure and its systematic implications in *Lamiaceae* have been thoroughly investigated by Ryding (1992, 1993a, b, 1994a, b, c).

In this study, transverse sections from nutlets of 19 species of the genus *Salvia* in Jordan have been prepared (Table 1). A few of the species do not occur in Jordan, such as *Salvia verticillata*, *S. bracteata*, *S. viscosa*. Differences in seed-coat layers (Table 2) and mucous production have been investigated (Table 3).

Table 1. Nutlet anatomy, thickness of pericarp, testa in micrometers (µm) and sclereid types.

Taxon	Epicarp	Mesocarp	Endocarp	Sclereid	Size of sclereids lumen	Testa
Salvia lanigera Poir.	13	25	30	mac-br.	10 x 8	50
S. multicaulis Vahl subsp. *multicaulis*	75	30	55	mac-br.	30 x 10*	UN
S. judaica Boiss.	50	38	38	mac-br.	18 x 10	5-8
S. verticillata L.	50	38	38	mac-br.	18 x 10	5-8
S. aegyptiaca L.	25	50	30	macro	5 x 5	10
S. bracteata Banks & Sol.	33	63	75	macro	UN	12.5
S. deserti Decne	25	25	38	macro	3.75 x 5	2.5
S. dominica L.	25	20	25	macro	10 x 10*	2
S. palaestina Benth.	25	50	25	macro	12.5 x 7.5	8
S. syriaca L.	25	25	25	macro	12.5 x 7.5*	13
S. verbenaca L.	75	25	35	macro	12.5 x 7.5*	13
S. viridis L.	15	28	18	mac-br.	12.5 x 7.5	13
S. viscosa Jacq.	5	13	30	mac-br.	12.5 x 12.5	5
S. ceratophylla L.	75	35	38	mac-br.	20 x 10	5
S. indica L.	25	35	100	mac-br.	75 x 13	8
S. spinosa L.	75	35	100	mac-br.	63 x 13	UN
S. eigii Zohary	75	25	35	mac-br.	13 x 8*	25
S. hierosolymitana Boiss.	75	25	35	mac-br.	13 x 8*	25
S. fruticosa Mill.	75	12.5	150	mac-br.	75 x 38*	UN

* sclereids with large lumen, UN = un-recognized.

Anatomical descriptions are presented with particular reference to the seed coat and pericarp for each species. A general diagram of the components of the nutlet coat is given in Fig. 1 and a detailed drawing for *Salvia* nutlets is provided in Fig. 2. Most of the *Salvia* species recorded in Jordan have been tested for mucilage production.

All the samples tested have given a positive test for mucilage production (Table 3). Measurements of epicarp, mesocarp, endocarp, type of sclereids and size of lumina as well as thickness of testa are also given in this Table.

Material and Methods

Wax embedding

The method used is that of Johansen (1940).

Spurr embedding medium

Spurr medium was tried and found to be unreliable, although the huge amount of mucous produced by the nutlets that makes the handling of the specimens difficult was not avoided.

Table 2. List of specimens studied.

Taxon	No.	Collector	Herbarium
Salvia multicaulis subsp. *multicaulis*	11999	D. Al-Eisawi	AMM
S. bracteata	3251	Hardjin	G
S. spinosa	477	M. Syoof	AMM
S. dominica	7143	L. Boulos & al.	AMM
S. ceratophylla	s.n.	D. Al-Eisawi	AMM
S. deserti	39	R. Jayusi	AMM
S. hierosolymitana	s.n.	S. A.Oran	AMM
S. verbenaca	8070	L. Boulos & al.	AMM
S. napifolia	1353	D. Al-Eisawi	AMM
S. indica	136	E. Akal	AMM
S. lanigera	2420	D. Al-Eisawi	AMM
S. viridis	52	A. Khalili	AMM
S. palaestina	11132	D. Al-Eisawi	AMM
S. syriaca	s.n.	D. Al-Eisawi	AMM
S. judaica	1559	Blanche	G
S. eigii	s.n.	Lowne	G
S. fruticosa	169	Amdursky	K
S. aegyptiaca	167	J. Audeh & al.	G
S. viscosa	172	Dleyn	G

AMM = Amman, G = Geneve, K = Kew.

Also at the sectioning stage, the ultra microtome used was not able to give complete sections probably because the diamond knife was not hard enough to cope with the hardness of the seed coat.

Table 3. List of nutlet specimens studied (mucilage production).

Taxon	Nutlet size (mm)	Colour	Shape	Results (mucilage)
Salvia aegyptiaca	2 x 1 - 3 x 2	black	ovoid	+
S. deserti	2 x 1 - 5-2.2	black	ovoid	+
S. bracteata	3.5 x 3 - 5 x 5	light brown	spheroidal	+
S. ceratophylla	3 x 3	black	spheroidal	+
S. dominica	2.5 x 2 - 5 x 3	light brown	ovoid	+
S. hierosolymitana	2.5 x 2.5	dark brown	spheroidal	+
S. indica	4.5 x 2.5	black	ovoid	+
S. lanigera	2.5 x 2	black	ovoid	+
S. multicaulis subsp. *multicaulis*	4 x 4	light brown	ovoid	+
S. napifolia	2.5 x 1.5	dark brown	ovoid	+
S. palaestina	4 x 3	glossy brown	ovoid	+
S. spinosa	3 x 2.7	light brown	rounded	+
S. verbenaca	2.5 x 2	black	ovoid	+
S. viridis	4 x 2 - 5 x 2	brown	ovoid	+
S. syriaca	3 x 2	light brown	spheroidal	+

1. *Pre-fixation.* The nutlets were boiled in water for half an hour, left to dry, then placed in vials containing 5% gluteraldehyde and kept overnight at room temperature.

2. *Washing.* The materials were washed 3 times each for two hours (soaking gently) with a buffer Sodium Cacoddylate.

3. *Post fixation.* The samples were then fixed with osmium tetraxide for 2 hours at 4°C, then washed as in step 2.

4. *Dehydration.* The materials were passed through a series of Acetone concentrations: 30%, 50%, 70% for 15 minutes, and 95% for 30 minutes, then in absolute Acetone twice for 30 minutes.

5. *Infiltration.* This step was carried out by using plastic media (Spurr's medium). This medium has been changed 4 times, the first change medium after 4-5 hours, then after one day each time. This step was carried out in the fumehood, using gloves and protective musk.

6. *Embeding.* The embeding was done by pouring all the material in a Spurr's media capsules. The specimens were oriented by using flat embedding modules. The capsules or the modules were kept in the oven for 8 hours for one day.

7. *Sectioning.* The capsules containing the nutlets were sectioned using an Surval Mt-2B ultramicrotome.

8. *Staining and mounting.* The sections were laid on a clean slide by using a fine camel-hair brush, one drop of methylene blue was added to the slide which was placed on a hot plate for few seconds before being washed carefully with water. The sections were mounted using D.P.X. and left to dry.

Results

Nutlet anatomy

The results show that the seed coat or the pericarp of the nutlets is made of the following layers:

I. *Epicarp.* This consists of the epidermis and the hypodermis layers. The transverse sections show an unclear or undifferentiated pericarp. The thickness of the epicarp for the specimens examined is significant because it varies from one taxon to another, between 5-75 µm as shown in Table 2.

II. *Mesocarp.* This layer is made up of an undifferentiated parenchymatous tissue and sometimes a single layer of columnar to tubular or pillar-like cells. Often these cells are separated from the epicarp layer and look shorter or un-connected with the upper layer.

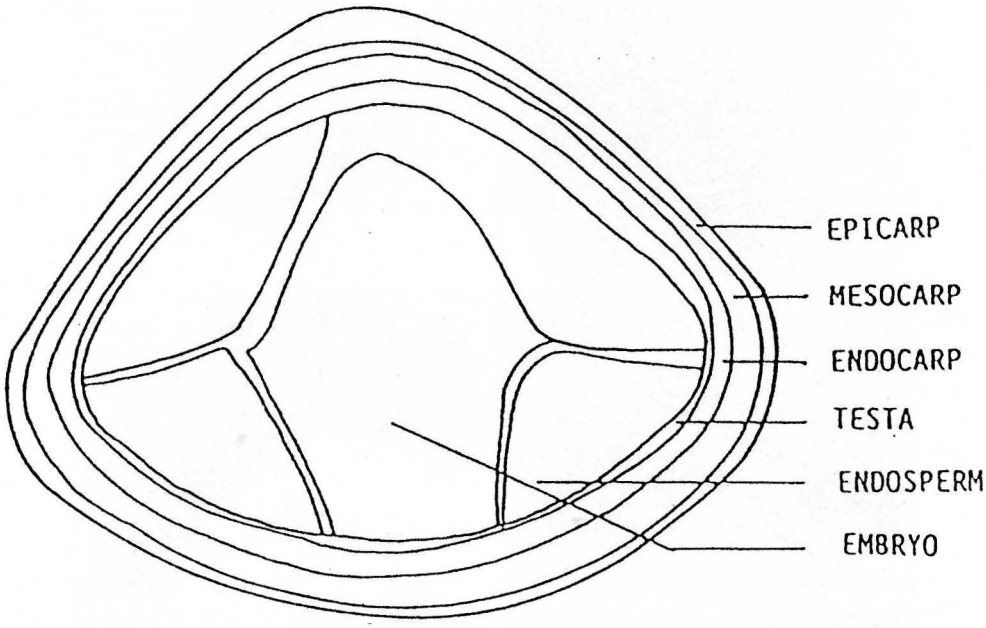

Fig. 1. Outline of *Salvia* seed cross section.

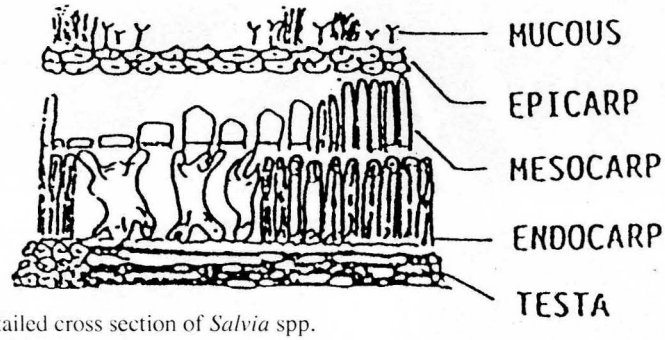

Fig. 2. Seed detailed cross section of *Salvia* spp.

III. *Endocarp*. The endocarp consists mainly of sclerenchymatic tissue, either columnar (macrosclerides) or with osteosclereids.

IV. *Testa*. This is the tissue which envelops the endosperm and the embryo. In the case of the sections studied here the testa has been ruptured either partially or entirely probably because of its thickened layer. It is of 1-2 layers thick of undifferentiated or sometimes clear brachy-sclereids.

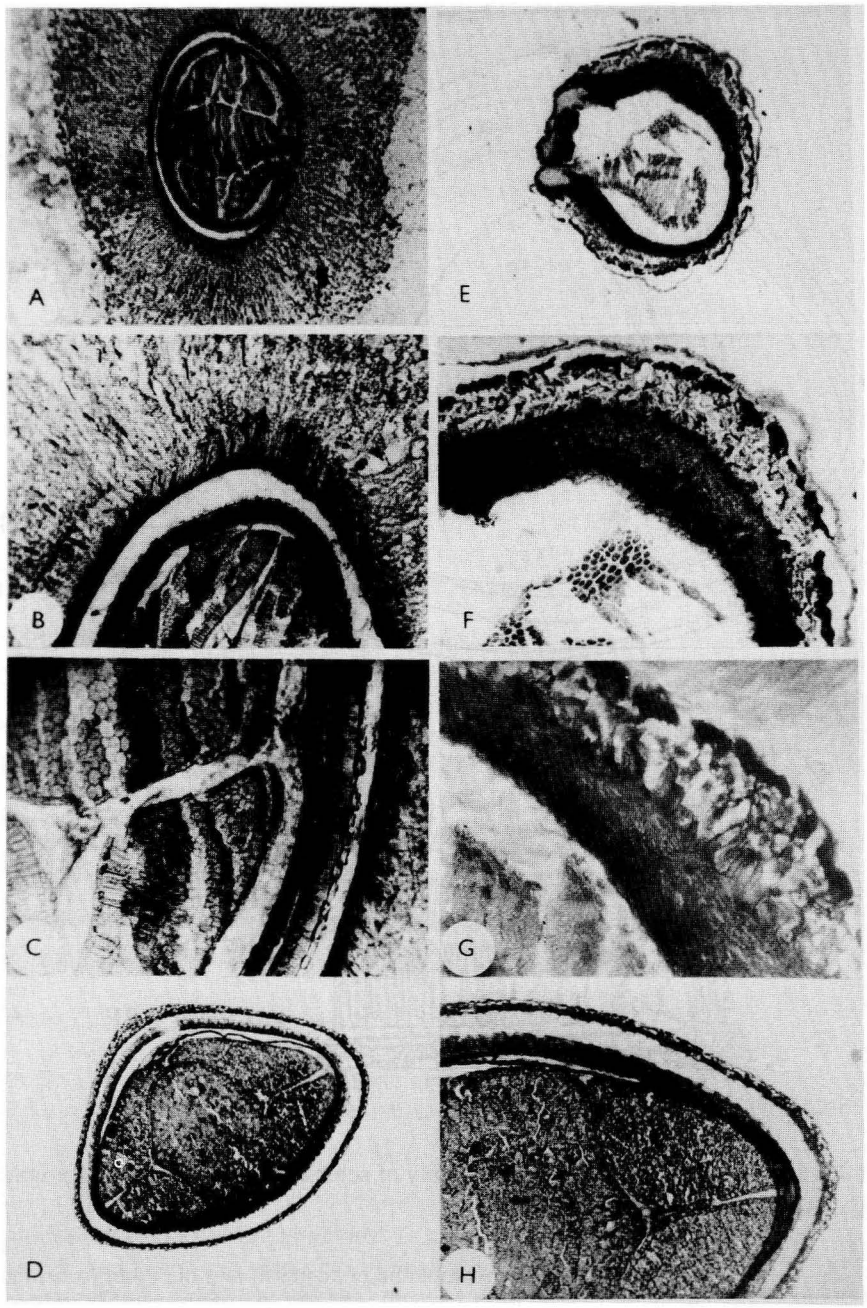

Fig. 3. Median transverse sections of the nutlets, showing the mucilage, pericarp (nutlet coat) components, testa and endosperm. **A,** *Salvia viridis* showing amount of mucilage (× 40); **B,** *S. viridis* (× 200); **C,** *S. viridis* (× 400); **D,** *S. aegyptiaca* (× 40); **E,** *S. bracteata* (× 40); **F,** *S. bracteata* showing un-differentiated epicarp and mesocarp (× 200); **G,** *S. bracteata* (× 200); **H,** *S. aegyptiaca* showing mucilage, epicarp, mesocarp lower part and minute (× 200).

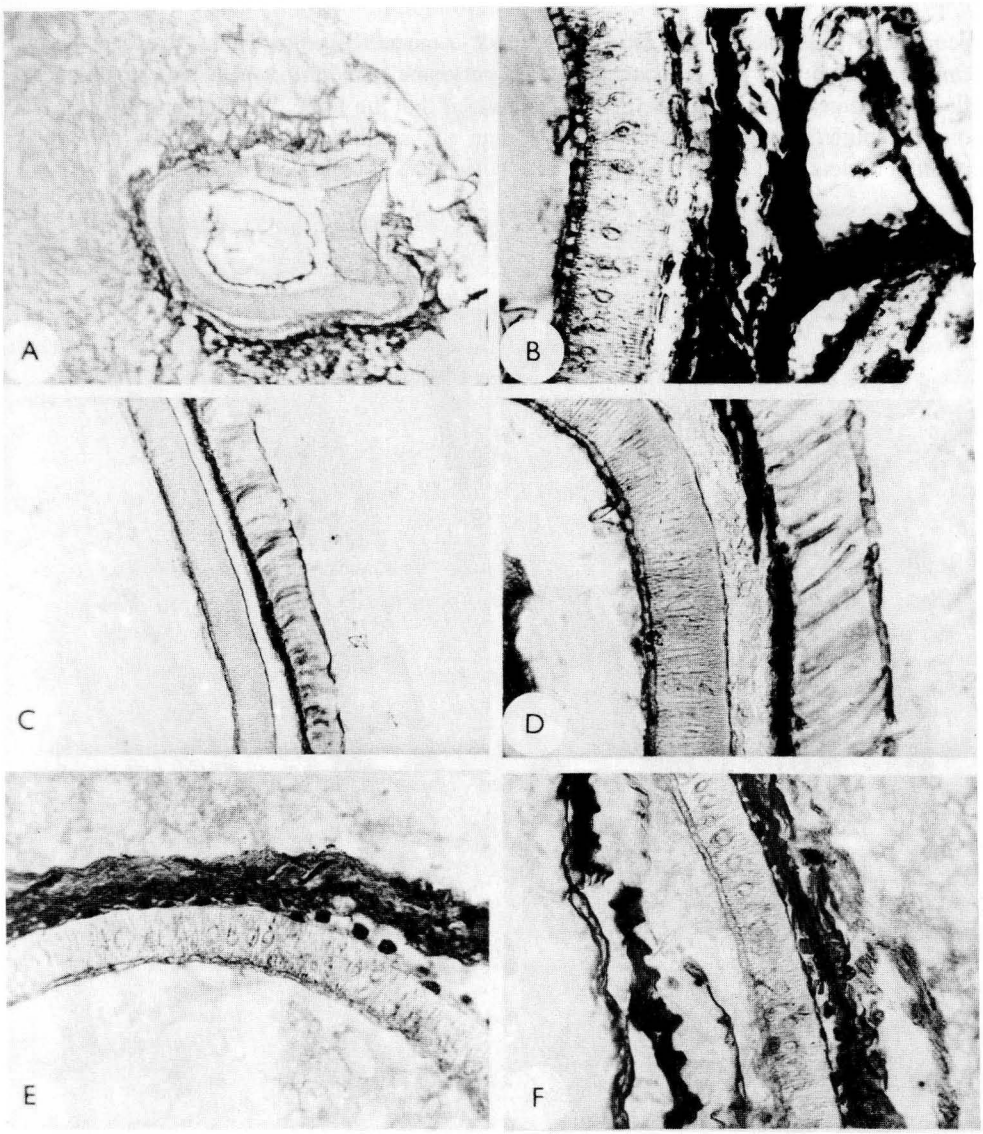

Fig. 4. Median transverse sections of the nutlets, showing the components of the pericarp (cell wall), testa and endosperm. **A,** *Salvia verbenaca* (× 100); **B,** *S. verbenaca* showing (un-differentiated mesocarp and large macrosclereids (× 200); **C,** *S. syriaca* (× 200); **D,** *S. syriaca* (× 200); **E,** *S. dominica* showing epicarp, thin mesocarp large macrosclereids (× 400); **F,** *S. dominica* (× 400).

Mucilage

14 species of the genus *Salvia* in Jordan have been tested for mucilage production by soaking the nutlets in water or boiling them for few minutes. As a result of wetting, the nutlets reacted by producing different amount of mucoid substances depending on the

species. All the nutlets tested seem to produce a mucous sheath (Table 3) except *Salvia napifolia* which only sometimes produced a very small amount or no visible mucilage at all. It has been noticed from the species examined that the large, rounded and light brown coloured nutlets produce more mucilage than the smaller, elliptical-shaped and black coloured nutlets. The greatest mucilage production was found in *S. viridis*.

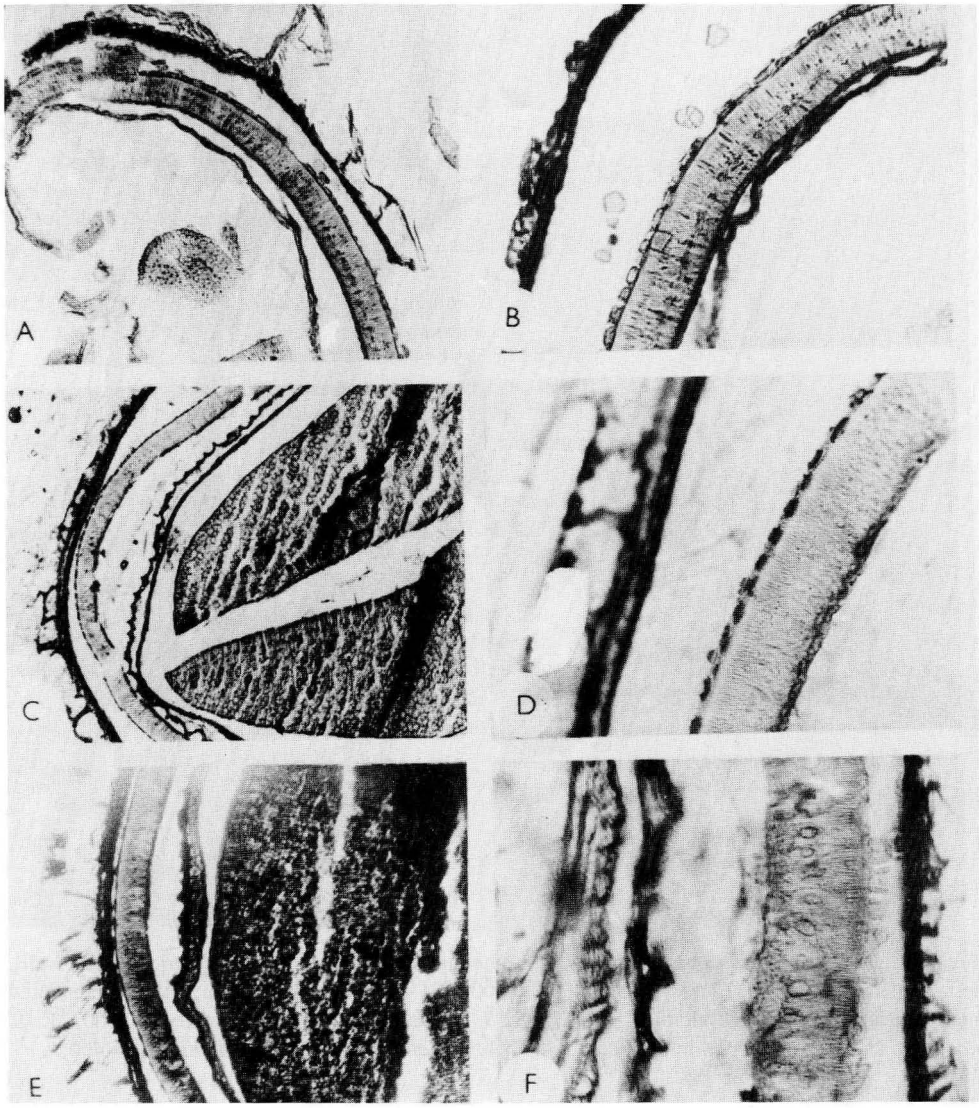

Fig. 5. Median transverse sections of the nutlets, showing mucilage, epicarp, mesocarp, testa and endosperm. **A,** *Salvia deserti* showing mucilage layer (ruptured), mesocarp, minute macrosclereids (× 200); **B**, *S. deserti* (× 400); **C,** *S. palaestina* showing mucilage layer, thin epicarp, mesocarp and minute macrosclereids (× 100); **D,** *S. palaestina* (× 400); **E,** *S. viscosa* (× 200); **F,** *S. viscosa* (× 400).

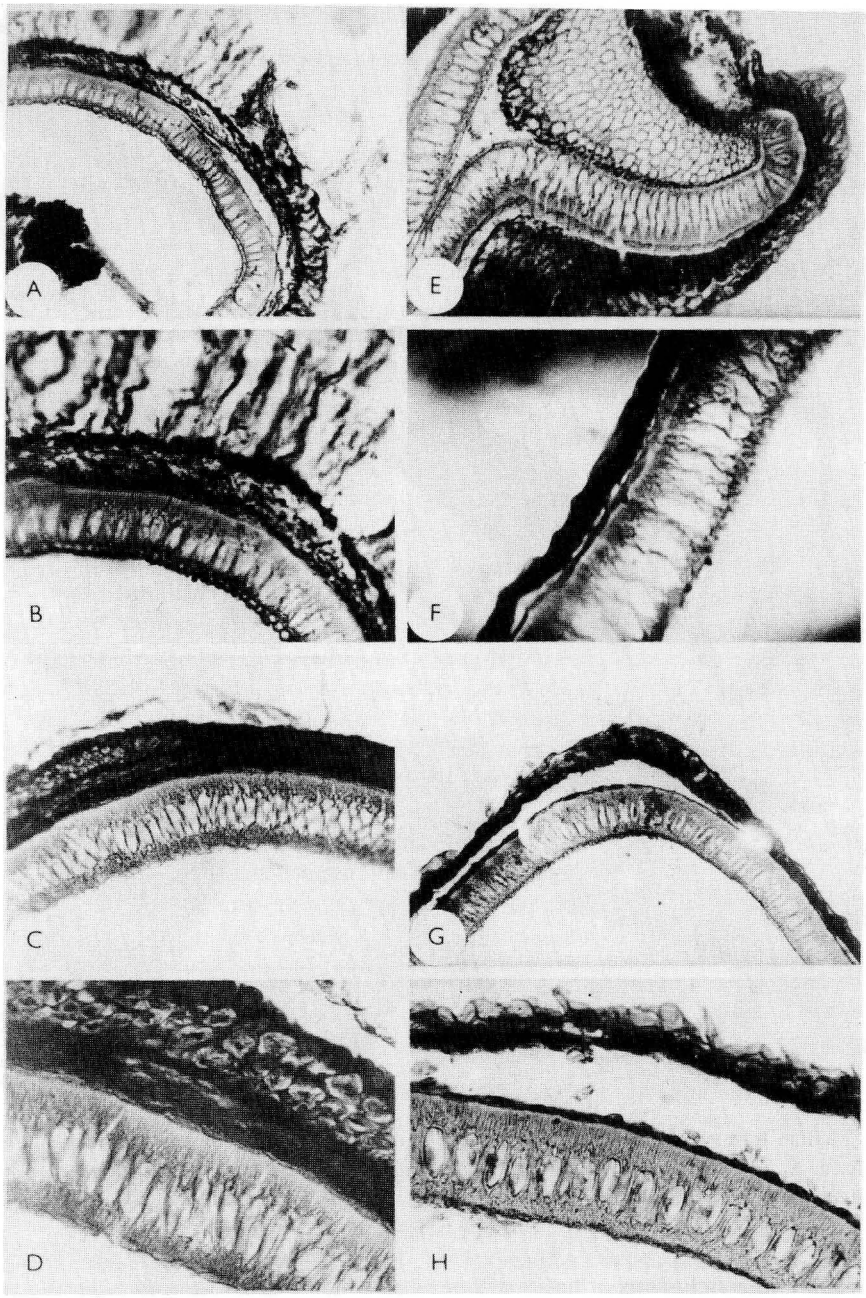

Fig. 6. Median transverse sections of the nutlets, showing mucilage, epicarp, mesocarp, testa and endosperm. **A,** *Salvia lanigera* (× 200); **B,** *S. lanigera* showing large osteosclereids and mucilage (× 400); **C,** *S. multicaulis* subsp. *multicaulis* (× 200); **D,** *S. multicaulis* subsp. *multicaulis* showing the cells of the epicarp and large osteosclereids (× 400); **E,** *S. judaica* (× 200); **F,** *S. judaica* (× 400); **G,** *S. napifolia* (× 200); **H**, *S. napifolia* (× 400).

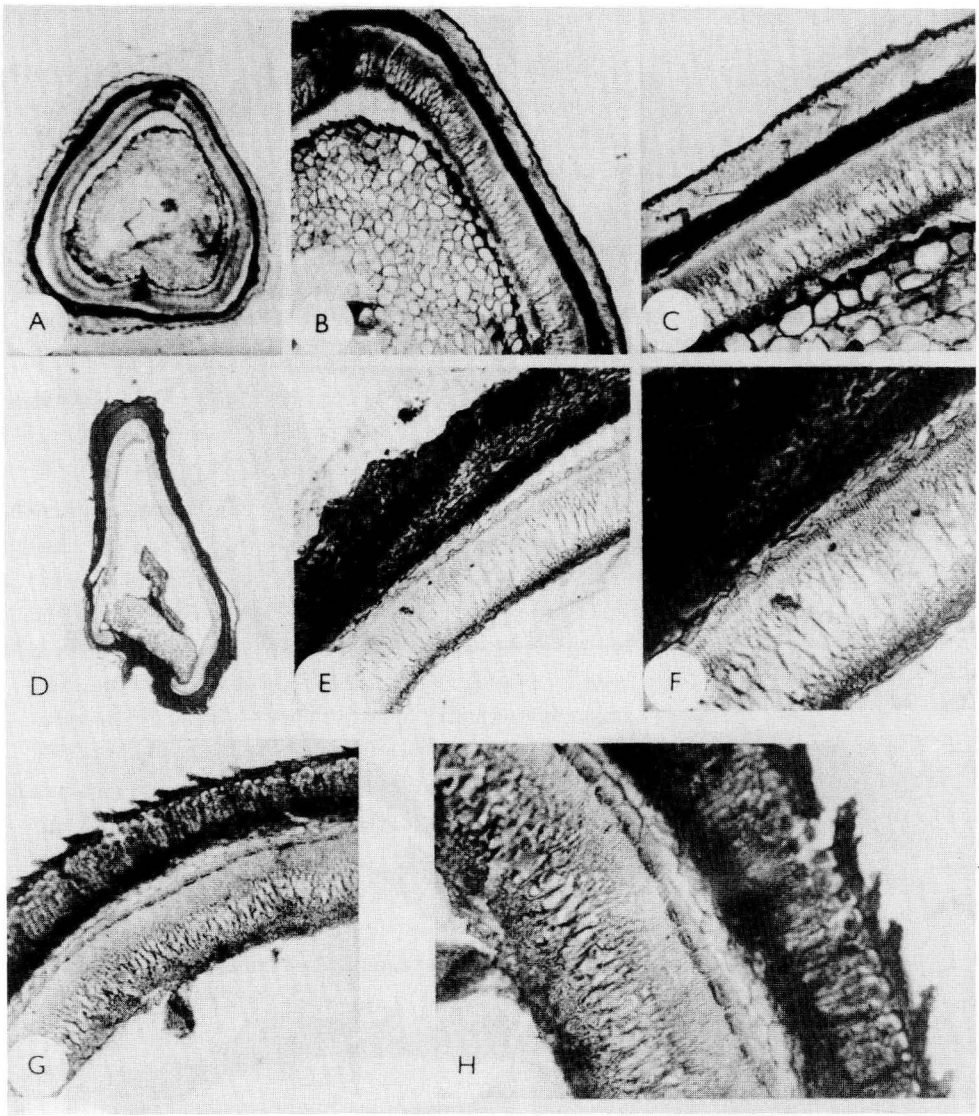

Fig. 7. Median transverse sections of the nutlets, showing mucilage, epicarp, mesocarp, testa and endosperm. **A,** *Salvia ceratophylla* (× 40); **B,** *S. ceratophylla* (× 200); **C,** *S. ceratophylla* (× 400); **D,** *S. indica* (× 40); **E,** *S. indica* (× 200); **F,** *S. indica* (× 400); **G,** *S. spinosa* (× 200); **H,** *S. spinosa* (× 400).

Discussion and conclusions

The anatomical results which have been obtained from studying the nutlet cross sections seem to have significantly discriminative taxonomic characters for the 19 species of *Salvia* studied. As far as the epicarp layer is concerned, it is found that the thickness of this layer is variable among the taxa studied, and thus, it is more useful taxonomically at

the sectional and specific levels. Also, the thickness of the mesocarp layer played a significant role in characterization of the taxa studied.

The thickness of this layer, as revealed in the results and as shown in Table 3 is variable among the different species which belong to different sections. The tissue components of the mesocarp layer have been found to some extent valuable. This layer has been recognized as an un-differentiated layer of parenchyma which appeared dark, or sometimes light, thick layers. It has been found variable among the sections and also even within the species of one section.

The columnar or tubular cells which have been recognized in cross section and which I believe are directly connected to the epicarp are sometimes separated from the epicarp and look as if they are of a different layer and appear shorter and transversely arranged. These cells have been considered by Hedge (1970) as a layer which is part of the mesocarp.

The most significant characters of the endocarp layer are its thickness, the sclerenchymatic tissue which has been recognized in the studied cross sections, and the size of the main lumen which varies in a taxonomically important way within the different species studied. Based on the type of sclereids present in the endocarp layer shown in each taxon studied, the following groups have been recognized:

Taxa with columnar (macrosclereids) and with relatively large lumina:

Salvia dominica	(Fig. 4E, F)
S. syriaca	(Fig. 4C, D)
S. verbenaca	(Fig. 4A, B)

Taxa with columnar (macrosclereids) and with minute lumina:

S. aegyptiaca	(Fig. 3D, H)
S. deserti	(Fig. 5A, B)
S. palaestina	(Fig. 5C, D)
S. viscosa	(Fig. 5E, F)
S. bracteata	(Fig. 3E, F, G)
S. viridis	(Fig. 3A, B, C)

Taxa with osteosclereids and small lumina:

S. multicaulis subsp. *multicaulis*	(Fig. 6C, D)
S. lanigera	(Fig. 6A, B)
S. judaica	(Fig. 6E, F) .
S. napifolia	(Fig. 6G, H)
S. indica	(Fig. 7D, E, F)
S. spinosa	(Fig. 7G, H)
S. ceratophylla	(Fig. 7A, B, C)

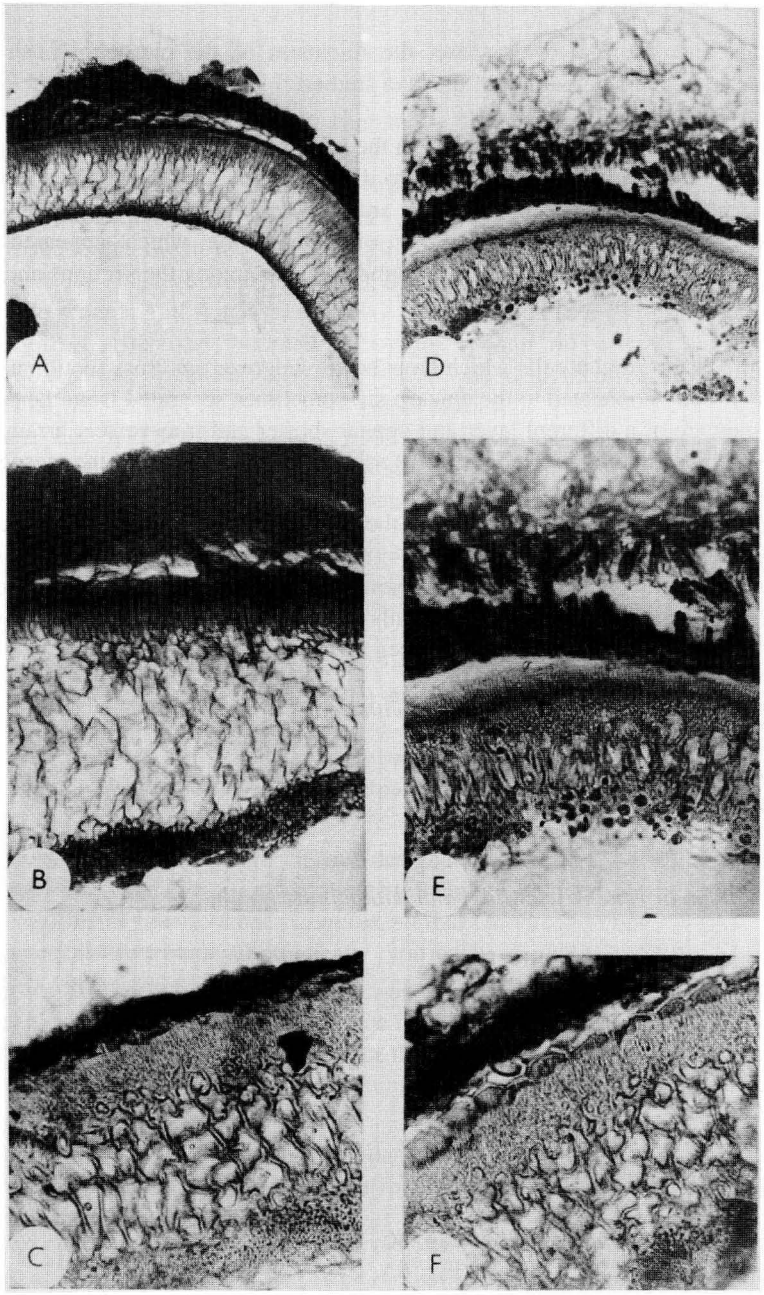

Fig. 8. Median transverse sections of the nutlets, showing mucilage, epicarp, mesocarp, testa and endosperm. **A,** *Salvia hierosolymitana* (× 200); **B,** *S. hierosolymitana* (× 400); **C,** *S. fruticosa* (× 200); **D,** *S. eigii* (× 200); **E,** *S. eigii* (× 200); **F,** *S. fruticosa* (× 400).

Taxa with osteosclereids and large lumina:

S. multicaulis subsp. *multicaulis*	(Fig. 6C, D)
S. fruticosa	(Fig. 8C, F)
S. hierosolymitana	(Fig. 8A, B)
S. eigii	(Fig. 8D, E)

The different types of sclereids in the endocarp layer of the fruits studied are significant and can be used as good taxonomic markers at the generic and specific levels. This has supported the idea of lumping the different taxa in this study, where it has been found that they have similar seed anatomical characters: *Salvia hierosolymitana* and *S. eigii, S. napifolia* and *S. judaica* etc.

The testa layer in most of the cross sections studied was ruptured possibly because of the rigidity of the layer and the presence of the hard brachysclereids. Therefore, the characteristics of this layer have not been very useful.

As far as mucilage production is concerned, all the taxa examined showed mucilage formation, although one species produced very little (Table 2). Mucilage has been discussed by Hedge (1968) in a study dealing with the mucilage of fruits in Afghanistan Salvias. He found that "There are appreciable differences between many species and their mucilage features". Therefore, this provides an extra taxonomic character. All but one of the taxa examined varies within a single habitat. Therefore other factors could be responsible for the formation of the mucilage rather than the habitat itself, and some of these probably the size, colour and the shape of the nutlets, soil quality, also the cell wall structure or may be it is a genetically fixed character of the species which needs more studies to explain this phenomenon.

In conclusion, the anatomical characteristics of some of the taxa studied confirm their similarity or close affinity with each other. The nutlet anatomy of *Salvia hierosolymitana* has similar anatomical structure to that of *S. eigii* sensu Zohary. *S. judaica* (Fig. 4E, F) has been found to have similar anatomical characteristics to *S. napifolia* (Fig 4G, H). This supports the lumping of the species into one taxon, *S. napifolia*. The nutlet coat and pericarp characteristics of some Salvias can be taxonomically useful at the generic and specific levels and the nutlets may be further supported if more characteristics are studied such as mucilage, endosperm and embryo.

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