Anatomical Observations on Nutlets of Some Salvia Species (Lamiaceae) from West Azarbaijan in Iran

Fatemeh Nejad Habibvash, Mohammad Ali Rajamand, Siavash Hosseini Surghhein, Reza Heidari and Mahnaz Heidari Rigan

Department of Biology, Faculty of Science, Urmia University, Urmia, West Azarbaijan, Iran

Agricultural Research Center of West Azarbaijan Herbarium, Iran

Abstract: Present survey was performed on thirteen species of Salvia from West Azarbaijan in Iran with the aim of illustrating species interrelationships. It includes comparative anatomy of the species based on nutlets transverse sections. These sections were examined using light microscope and detailed description of nutlet anatomical feature for all examined taxa is provided. In this study its found that the nutlets showed a considerable uniformity from anatomical point of view. However these species can be differentiated based on thickness of pericarp layer and parenchymatous layers colour.

Key words: Salvia, Lamiaceae, pericarp anatomy, Iran

INTRODUCTION

The family Lamiaceae includes 200 genera and 3000 species. The genus Salvia L. with over 900 species is probably the largest member of the family Lamiaceae. The two largest centres of the genus are in America and in South-West Asia (Hedge, 1992). Anatolia is a major centre for Salvia L. where 50.6% of the 87 species are endemic in Turkey (Vural and Adigüzel, 1996). Fifty-eight species of the genus Salvia (Lamiaceae) are found in Iran of which 17 are endemic. The rate of endemism in the Salvia in Iran is ca. 29% (Mozaffarian, 1996). In recent studies on this plant species it has been observed that the compounds decrease DNA synthesis in the cell. This feature is important in the diagnosis and treatment of cancer (Nakiboglu, 1993). A gelatinous substance is produced from the seed that has mucilage. This substance is used as a good varnish and sweetener in Mexico (Estila and Hashemi, 1990).

Species of the genus Salvia L. have high hybridization rate (Haq, 1981).

Since most of the genus Lamiaceae are important in medical and economic terms the members of the family need to be reviewed in terms of their systematic positions. Therefore some new revisions and studies have been carried out to elucidate the morphological, anatomical and chemical characters of the family members (Nakiboglu, 2002). The structure of the conductive bundles in the cross-section of the leaf petiols of the Lamiaceae members may be useful taxonomically (Metcalfe and Chalk, 1950).

Numbers of the conductive bundles in the cross-sectioned petiols of the Salvia L. species varied among the species (Nakiboglu and Oguz, 1990). Anatomy of the stems have shown that the numbers of rays in every species can be used as a species-distinguishing feature (Ozdemir and Serel, 1999).

Studies on nutlets in the Lamiaceae have proved taxonomic hierarchy. Ryding (2001) and Hye-Kyoung and Hong (2006) in a series of papers have made a detailed study of pericarp structure in Lamiaceae. Pericarp anatomical studies of some species of Salvia has been examined by some researchers (Wagner, 1914; Wojciechowska, 1961; Hedge, 1970). There are almost no reports on the pericarp anatomy of the species found in Iran. The aims of the present study are to offer a proved detailed description of the pericarp structure of the genus Salvia and to indicate species interrelationships.

MATERIALS AND METHODS

We initiated the anatomical studies in the Laboratory of Anatomy and Morphology in Biology Department of Urmia University with living materials which were collected from their natural habitats in West Azarbaijan in Iran during mid May and end of June 2006.

For pericarp anatomical studies dry nutlets were placed for 10 days in a mixture of distilled water, 96% ethanol and glycerol taken in equal proportions. In the middle part of nutlets 8 μm thick cross-sections were made using a rotary microtome. The paraffin method was used
Table 1: Anatomical measurements on *Salvia* species

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Mean thickness of endocarp (μm)</th>
<th>Mean thickness of sclerenchymatous cell (μm)</th>
<th>Mean thickness of mesocarp (μm)</th>
<th>Mean thickness of mucilage (μm)</th>
<th>Mean thickness of cuticle (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. multiflorus</em> VAHI</td>
<td>3.00±0.00</td>
<td>1.22±0.22</td>
<td>0.66±0.16</td>
<td>1.17±0.10</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td><em>S. ceratophylla</em> L</td>
<td>3.16±0.60</td>
<td>3.09±0.10</td>
<td>3.66±0.33</td>
<td>1.42±0.08</td>
<td>0.24±0.08</td>
</tr>
<tr>
<td><em>S. nemorosa</em> L</td>
<td>2.00±0.48</td>
<td>1.14±0.05</td>
<td>0.50±0.00</td>
<td>1.54±0.02</td>
<td>0.26±0.09</td>
</tr>
<tr>
<td><em>S. artemisia</em> Bunge</td>
<td>7.16±0.61</td>
<td>2.18±0.14</td>
<td>4.33±0.66</td>
<td>0.98±0.02</td>
<td>0.33±0.07</td>
</tr>
<tr>
<td><em>S. cardifolia</em> VAHI</td>
<td>2.66±0.44</td>
<td>1.98±0.08</td>
<td>0.75±0.00</td>
<td>1.65±0.14</td>
<td>0.25±0.03</td>
</tr>
<tr>
<td><em>S. virga</em> A.C.Q.</td>
<td>1.83±0.16</td>
<td>1.41±0.11</td>
<td>1.16±0.16</td>
<td>1.17±0.11</td>
<td>0.10±0.04</td>
</tr>
<tr>
<td><em>S. sclarea</em> L</td>
<td>2.00±0.09</td>
<td>1.58±0.14</td>
<td>0.91±0.08</td>
<td>1.52±0.00</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td><em>S. verticillata</em> L</td>
<td>1.83±0.16</td>
<td>1.04±0.12</td>
<td>0.85±0.16</td>
<td>1.33±0.22</td>
<td>0.40±0.12</td>
</tr>
<tr>
<td><em>S. syriaca</em> L</td>
<td>2.00±0.00</td>
<td>1.49±0.02</td>
<td>0.91±0.08</td>
<td>2.24±0.00</td>
<td>0.29±0.09</td>
</tr>
<tr>
<td><em>S. macrophylla</em> BOISS</td>
<td>3.66±0.33</td>
<td>2.90±0.25</td>
<td>3.00±0.00</td>
<td>3.76±0.40</td>
<td>1.04±0.16</td>
</tr>
<tr>
<td><em>S. oerbii</em> L</td>
<td>3.33±0.33</td>
<td>1.52±0.12</td>
<td>0.75±0.14</td>
<td>2.18±0.07</td>
<td>0.13±0.02</td>
</tr>
<tr>
<td><em>S. Hydrangea DC. ex Roth</em></td>
<td>0.91±0.08</td>
<td>1.54±0.26</td>
<td>0.75±0.00</td>
<td>1.84±0.00</td>
<td>0.21±0.05</td>
</tr>
<tr>
<td><em>S. urmerica</em> BLUNER</td>
<td>2.00±0.00</td>
<td>1.28±0.04</td>
<td>0.50±0.00</td>
<td>0.85±0.07</td>
<td>0.25±0.08</td>
</tr>
</tbody>
</table>

Table 2: Analysis of variance for parameters in *Salvia* species

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>cl</th>
<th>ml</th>
<th>pl</th>
<th>sl</th>
<th>dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>12</td>
<td>0.172</td>
<td>1.714</td>
<td>5.137</td>
<td>1.277</td>
<td>7.472</td>
</tr>
<tr>
<td>Within groups</td>
<td>26</td>
<td>0.020</td>
<td>0.040</td>
<td>0.155</td>
<td>0.068</td>
<td>0.354</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

c: cuticular layer, m: mucilaginous layer, p: parenchymatous layer, s: sclerenchymatous layer, e: endocarp layer

Table 3: The localities of plant species

<table>
<thead>
<tr>
<th>Taxon</th>
<th>The localities of plant species</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. multiflorus</em> VAHI</td>
<td>Naghash; Solian Yaghchal Valley; Oshnavieh; Badran Zard; Urmia; Shohada Valley; Khan Valley; Oshnavieh road; Urmia; Nooshinshahr; Keshish Mountain</td>
</tr>
<tr>
<td><em>S. ceratophylla</em> L</td>
<td>Urmia; Gharebagh Village; Urmia; between Gharebagh and Agzirin Village</td>
</tr>
<tr>
<td><em>S. nemorosa</em> L</td>
<td>Urmia; Shohada Valley; Chaldan Salma; between Koozehnahr and Sarichchak Village; Maku; Siahcheshmeh</td>
</tr>
<tr>
<td><em>S. artemisia</em> Bunge</td>
<td>Urmia; Gholoohi Mountains; Oshnavieh Road</td>
</tr>
<tr>
<td><em>S. cardifolia</em> VAHI</td>
<td>Urmia; Marmisho</td>
</tr>
<tr>
<td><em>S. virga</em> A.C.Q.</td>
<td>Khoy; Grese Village and road of Gherzezianidin</td>
</tr>
<tr>
<td><em>S. sclarea</em> L</td>
<td>Maku; Siahcheshmeh; Urmia; Nazlou Village; Urmia; Shamlakan Village; Khoy; 25 km of road of Gharezianidin; Sarashi; 25 km of Piranshahr</td>
</tr>
<tr>
<td><em>S. verticillata</em> L</td>
<td>Urmia; Gholoohi Mountains; Urmia; Marmisho; Maku; Bazargan</td>
</tr>
<tr>
<td><em>S. syriaca</em> L</td>
<td>Urmia; Gholoohi Mountains; Oshnavieh; Mir Abad Jungle</td>
</tr>
<tr>
<td><em>S. macrophylla</em> Boiss. and kotschyi</td>
<td>Urmia; Marmisho</td>
</tr>
<tr>
<td><em>S. oerbii</em> L</td>
<td>Khoy; Greece Village</td>
</tr>
<tr>
<td><em>S. Hydrangea DC. ex Roth</em></td>
<td>Urmia; Kaboodan Island; Maku; Siahcheshmeh; Gharezianidin Road</td>
</tr>
<tr>
<td><em>S. urmerica</em> BLUNER</td>
<td>Naghash; Solian Yaghchal Valley</td>
</tr>
</tbody>
</table>

for preparing a cross section of nutlets and these cross-sections were stained with Hematoxylin-Eosin (Johnson, 1940). Suitable sections were taken from these materials for microscopic studies. The thirteen species of *Salvia* growing in West Azarbaijan were selected as study materials (Table 3). The thickness of the layers in the nutlets was measured micrometrically and the results are given in Table 1. All the chemicals were purchased from Merck, Germany.

Values are expressed as means ± SE of three replications. For statistical analysis, one-way analysis of variance followed by ANOVA and Tukey's test were used. p<0.05 was considered significant (Table 2).

RESULTS AND DISCUSSION

The results obtained from the pericarp anatomical studies are presented (Table 1, 2 and Fig. 1).

The basic structure of pericarp layer was found to be uniform in all investigated taxa but there was clear differences in thickness of pericarp layer and parenchymatous layer's colour. In all the taxa studied the thickness of cuticular layer is 0.08-1.36 μm and that of mucilaginous layer is 0.25-4.24 μm. In the middle of pericarp structure there are layers of yellowish, brown, dark-brown or black parenchymatous cells with thickness from 0.5 to 4 μm. Trackid groups inside these layers have a sheath of elongated parenchymatous cells around them. The upper cell layers of the mesocarp are broader than the innermost ones. In all investigated taxa the sclerenchymatous layer of the endocarp has a smooth sculpture with 0.8-3.2 μm thickness. The cells in the sclerenchymatous region consist of a layer of vertically arranged bone cells. There is a star-like cavity which is enlarged and branched at the centre, this cavity has pit canals towards the apex as well as the base. In all investigated taxa thickness of endocarp layer is between 0.75 and 8 μm.

*S. multiflorus* VAHI: The thickness of cuticular layer is 0.08-0.16 μm and that of the mucilaginous layer is 0.96-1.28 μm.
Fig. 1: Fecarip transverse sections of *Satvia* species

A: *S. hydrangea* Scale bar: 25 µm
B: *S. aropotana* Scale bar: 25 µm
C1, C2: *S. multicaulis* Scale bar: 25 µm
C3: Details of sclerenchymatous layer Scale bar: 10 µm
D: *S. ceratophylla* Scale bar: 25 µm
E: *S. sclarea* Scale bar: 25 µm
F: *S. verticilata* Scale bar: 25 µm
G: *S. candidissima* Scale bar: 25 µm
H: *S. macrochamps* Scale bar: 25 µm
I: *S. ethiopia* Scale bar: 25 µm
J: *S. urminensis* Scale bar: 25 µm
K: *S. nemorosa* Scale bar: 25 µm
L: *S. syriaca* Scale bar: 25 µm
M, N: *S. virgate* Scale bar: 25 µm
N: Details of paracymatous layer

Cl: Cuticle layer
mc: Mucilaginous cell
pl = Paracymatous layer
sl = Sclerenchymatous layer
Sc = Seedcoat
In the centre of the pericarp structure there are 0.5-1 µm thick, dark-brown parenchymatous layer, under the mesocarp layer there are bone cells with 0.9-2.56 µm and endocarp layer is 3 µm thick.

S. ceratophylla L.: The thickness of cuticular layer is 0.08-0.32 µm and that of the mucilaginous layer is 1.32-1.6 µm. In the centre of the pericarp structure, there are 3-4 µm thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 2.88-3.2 µm and endocarp layer is 3-5 µm thick.

S. nemorosa L.: The thickness of cuticular layer is 0.08-0.32 µm and that of the mucilaginous layer is 1.52-2.24 µm. In the centre of the pericarp structure, there are 0.5 µm thick, dark-brown parenchymatous layers, under the mesocarp layer there are bone cells with 1.04-1.2 µm and endocarp layer is 1-3 µm thick.

S. atropatana Bunge: The thickness of cuticular layer is 0.24-0.48 µm and that of the mucilaginous layer is 0.96-1.4 µm. In the centre of the pericarp structure, there are 3-5 µm thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 1.92-2.4 µm and endocarp layer is 6.8 µm thick.

S. candidissima Vahl: The thickness of cuticular layer is 0.2-0.32 µm and that of the mucilaginous layer is 1.44-1.92 µm. In the centre of the pericarp structure, there are 0.75 µm thick, black parenchymatous layers, under the mesocarp layer there are bone cells with 1.88-2.16 µm and endocarp layer is 2-3.5 µm thick.

S. virgata Jacq: The thickness of cuticular layer is 0.08-0.16 µm and that of the mucilaginous layer is 0.96-1.36 µm. In the centre of the pericarp structure, there are 1 µm thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 1.2-1.6 µm and endocarp layer is 1.5-2 µm thick.

S. sclarea L.: The thickness of cuticular layer is 0.16-0.24 µm and that of the mucilaginous layer is 1.52 µm. In the centre of the pericarp structure, there are 0.75-1 µm thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 1.36-1.84 µm and endocarp layer is 2 µm thick.

S. vertisillata L.: The thickness of cuticular layer is 0.24-0.64 µm and that of the mucilaginous layer is 0.88-1.6 µm. In the centre of the pericarp structure, there are 0.5-1 µm thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 0.8-1.2 µm and endocarp layer is 1.5-2 µm thick.

S. syriaca L.: The thickness of cuticular layer is 0.16-0.48 µm and that of the mucilaginous layer is 0.96-1.36 µm. In the centre of the pericarp structure, there are 1 µm thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 1.44-1.52 µm and endocarp layer is 1.52-2 µm thick.

S. macrochlamys Boiss: The thickness of cuticular layer is 0.9-1.36 µm and that of the mucilaginous layer is 3.53-4.24 µm. In the centre of the pericarp structure, there are 3 µm thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 2.4-3.2 µm and endocarp layer is 3-4 µm thick.

S. aethiopis L.: The thickness of cuticular layer is 0.08-0.16 µm and that of the mucilaginous layer is 2.32-2.8 µm. In the centre of the pericarp structure, there are 0.5-1 µm thick, brown parenchymatous layers, under the mesocarp layer there are bone cells with 1.28-1.68 µm and endocarp layer is 2-3 µm thick.

S. hydrangea DC. ex Benth: The thickness of cuticular layer is 0.16-0.32 µm and that of the mucilaginous layer is 0.25 µm. In the centre of the pericarp structure, there are 0.75 µm thick, dark parenchymatous layers, under the mesocarp layer there are bone cells with 0.75-1 µm and endocarp layer is 1.28-2.08 µm thick.

S. urmiensis Bunge: The thickness of cuticular layer is 0.12-0.4 µm and that of the mucilaginous layer is 0.72-0.96 µm. In the centre of the pericarp structure, there are 0.5 µm thick, yellowish parenchymatous layers, under the mesocarp layer there are bone cells with 1.2-1.36 µm and endocarp layer is 2 µm thick.

The cuticular layer: Analysis of species average comparison variance conducted with ANOVA (p<0.05) revealed that there is a significant difference about the cuticular layer thickness among species also Tukey's test showed that species S. hydrangea, S. atropatana, S. multicaitis, S. ceratophylla, S. sclarea, S. vertisillata, S. candidissima, S. aethiopis, S. urmiensis, S. nemorosa, S. syriaca, S. virgata are placed in a homogenous class and S. macrochlamys is placed in a seconded class. The layer thickness difference among species belonging to the same class is not considerable.

The mucilaginous layer: Analysis of species average comparison variance conducted with ANOVA (p<0.05) and Tukey's test results showed that based on the mucilage thickness species are divided into six class: species S. urmiensis, S. atropatana, S. virgata, S. multicaitis, S. vertisillata, S. ceratophylla;

The mesocarp layer: Analysis of species average comparison variance conducted with ANOVA (p<0.05) and Tukey’s test results showed that based on the mesocarp layer thickness, species are classified into 3 classes: species S. urmiensis, S. nemorosa S. virgata, S. hydrangea, S. candidissima, S. aethiops, S. verticillata, S. sclarea, S. syriaca, S. virgata; S. macrochlamys, S. ceratophylla, S. atropatana, S. ceratophylla belong to the classes 1 to 3, respectively.

The sclerenchymatous layer: Analysis of species average comparison variance conducted with ANOVA (p<0.05) and Tukey’s test results showed that based on the sclerenchymatous layer thickness species are classified into 6 class: species S. hydrangea, S. sclarea, S. verticillata, S. nemorosa S. urmiensis, S. virgata, S. syriaca, S. aethiops, S. urmiensis, S. virgata, S. syriaca, S. aethiops, S. sclarea, S. hydrangea, S. candidissima; S. virgata, S. syriaca, S. aethiops, S. sclarea, S. hydrangea, S. candidissima, S. multicaulis, S. syriaca, S. sclarea, S. aethiops, S. hydrangea, S. candidissima, S. multicaulis, S. atropatana, S. syriaca, S. aethiops, S. sclarea, S. hydrangea, S. candidissima, S. multicaulis, S. atropatana, S. macrochlamys, S. ceratophylla belong to classes 1 to 6, respectively.

The endocarp layer: Analysis of species average comparison variance conducted with ANOVA (p<0.05) and Tukey’s test results showed that based on the endocarp layer thickness species are classified into five classes: class one consists of species: S. verticillata, S. virgata, S. urmiensis, S. sclarea, S. nemorosa S. syriaca, S. aethiops, S. candidissima; class two consists of species: S. verticillata, S. virgata, S. candidissima, S. urmiensis, S. nemorosa S. syriaca, S. aethiops, S. candidissima, S. multicaulis; class three consists of species: S. candidissima, S. multicaulis, S. macrochlamys, S. aethiops, S. syriaca, S. nemorosa S. urmiensis, class four consists of species: S. candidissima, S. multicaulis, S. macrochlamys, S. ceratophylla and class 5 consists of species S. atropatana.

In conclusion our results highly support the results given by other workers on other species of Salvia L. (Wagner, 1914; Wojciechowska, 1961; Hedge, 1970).

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REFERENCES


