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## MORPHOLOGICAL PECULIARITIES OF THE NUTLETS OF SOME *SALVIA* SPECIES (*LAMIACEAE*)

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**Abstract:** *Salvia*, the largest genus in the family Lamiaceae, includes species with different economic uses: medicinal, decorative, bee flowers, etc. In this paper we present new information on the nutlet morphology of 12 species of *Salvia*. The botanical material was collected from the Botanical Garden of Iași or from different parts of the country, and is represented by both wild and cultivated taxa.

The binocular magnifier and photonic microscope allow highlighting of characteristics of the testa as regards colour, size, presence and arrangement of the abscission scar, and the presence of ornamentation. Nutlet shape is ovoid or ellipsoidal and the surface displays colliculate, verrucose, reticulate or foveolate ornamentation. A rarely used index is the ratio of maximum diameter of the nutlets and maximum diameter of the abscission scar, with values 3–18.5. To illustrate the taxonomic value of this information, based on all the observed features, a key has been developed for identification of the 12 *Salvia* taxa studied, based on characters of the nutlets.

The phenomenon of myxocarpy, characteristic of Lamiaceae, revealed differences on the basis of its presence or absence, speed of occurrence, and consistency of the characteristic mucilage. As these observations differ from some data in the literature, explanation of some aspects requires further study.

This work complements the theoretical and detailed iconographic information in the literature about nutlets, which is useful for clarifying some aspects of the taxonomy of *Salvia*.

**Keywords:** nutlets, *Salvia*, seeds surface, myxocarpy.

### Introduction

*Salvia* is the largest genus in Lamiaceae and comprises an enormous, cosmopolitan group of some 1000 species of wide ecological amplitude. The greatest number of species (500) is found in Central and South America, 250 species occur in Central Asia and the Mediterranean region, and 90 in South-East Asia. In the Romanian flora, the genus is represented by both wild and cultivated species; lately increased interest has been shown in horticultural cultivars of species such as *Salvia coccinea*, *S. farinacea*, *S. officinalis*, *S. splendens* and *S. viridis*. Many taxa are well known for their use in classical or traditional medicine, cosmetics and/or aromatherapy, as bee flowers, etc.

Morpho-anatomical studies on the members of this genus are very numerous, but features of the nutlet have now begun to be investigated in more detail, especially with the improvement of modern techniques [2, 14].

In the Romanian literature, the most numerous and detailed morphological data on nutlets of the wild species (and those occasionally grown) of *Salvia* are, as expected, in *Flora R.P.R.* [7]. However, a closer look reveals that these data are incomplete, inadequate or even ambiguous.

In recent decades, the use of micro-morphological characters in the classification of Lamiaceae has expanded greatly [3]. The features of the seeds and the surface of the nutlets are helpful evaluative characters in systematic studies. These can be used successfully at several taxonomic levels, depending on the chosen character and its variation.

Some of the most valuable data obtained from the study of the pericarp structure and its implication in taxonomy can clarify the situation of some uncertain species or their generic relationships [5, 11, 20].

Contributions in the domain of Romanian authors are less numerous, but the techniques used to obtain information are varied, both classical [10, 22] and modern (e.g. SEM) [4, 16, 17].

This study aims to investigate the differences in ornamentation and mucilage production in *Salvia* nutlets in order to obtain new information, especially for the wild species.

### Material and Methods

The material studied is represented by the nutlets of 12 taxa from the genus *Salvia*. Most have been collected from the spontaneous flora of the Botanical Garden of Iași (*S. austriaca* Jacq., *S. nemorosa* L. subsp. *nemorosa*, *S. sclarea* L.) or are cultivated there (*S. coccinea* Buc'hoz ex Etl., *S. officinalis* L., *S. ringens* Sibth. & Sm., *S. tiliifolia* Vahl., *S. verbenaca* L., *S. verticillata* L., *S. viridis* L.). *Salvia aethiopsis* L. has been collected from the "Pădurea Hagieni" natural reserve (Constanța County) and *Salvia glutinosa* L. from Cașoca village (Siriu, Buzău County).

Parameters such as shape, colour, appearance and abscission scar disposition were observed with an Optika binocular microscope [13], while the ornamentation of the tests of the nutlets was observed with an Optika photonic microscope. The size of the nutlet and its abscission scar was measured for 12 samples using an optical micrometer. For all taxa the index derived from the ratio of the maximum diameter of the nutlet and the maximum diameter of the abscission scar was calculated.

In order to highlight the phenomenon of myxocarpy, nutlets were wetted in distilled water and maintained there for 30 minutes, after which they were analyzed using an Optika binocular microscope. We have focused on the following aspects: the presence or absence of mucilage and its consistency. The most relevant aspects showing features of the nutlets or myxocarpy have been photographed with a Canon A540 apparatus.

### Results

The schizocarpic fruit of *Salvia* species is here referred to as a *nutlet*, a term preferred in many recent works [8, 20, 23] to that of mericarp, as used by Özkan *et al.* [15]. Furthermore, in works on this topic published in Romania, usually the term nutlet is used in conjunction with members of the Lamiaceae [4, 7, 16, 17].

The results of the macro- and microscopic study are presented in Table 1. The colour of the nutlets is generally brown, uniform or uneven, with darker stripes (*S. aethiopsis*, *S. nemorosa*, *S. sclarea*, *S. viridis*) or a mottled appearance (*S. coccinea* and *S. tiliifolia*).

Nutlet shape (Fig. 1) varies from almost spherical (*S. officinalis*) to oval or elliptical. *Salvia ringens* is characterized by the irregular shape of the distal part of the nutlets, compared with the smooth appearance of all the other species.

On the reverse side of the nutlets the abscission scar can easily be seen. This term is the one preferred by Kubitzki *et al.* [8] or Özkan *et al.* [15], to "attachment scar", as used by Guerin [6]. Obviously, the term "hilum" used by Gușuleac in *Flora R.P.R.* [7] to describe members of Labiatae [Lamiaceae] refers to the same scar. For species of *Hemigenia* R.Br, and *Microcorys* R.Br, Guerin [6] has proposed seven distinguishing characteristics of the abscission scar. Thus, the size of the scar (which can be above or below half of the nutlet, according to Guerin) can also yield useful information for the taxonomy of the genus *Salvia*.

In this present work we have focused on the ratio between the size of the nutlet and the size of the scar, which turned out to be variable. The abscission scar has, in general, a circular form, with irregular variations, to an obvious triangular shape in *S. aethiopsis*.

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The terminology used to describe the seed surface observed with a photonic microscope is in accordance with the literature [12, 18]:

*colliculate*: having small raised eminences or rounded broad elevations, closely spaced, covering the whole seed coat,

*foveolate*: with small deep pits; finely pitted,

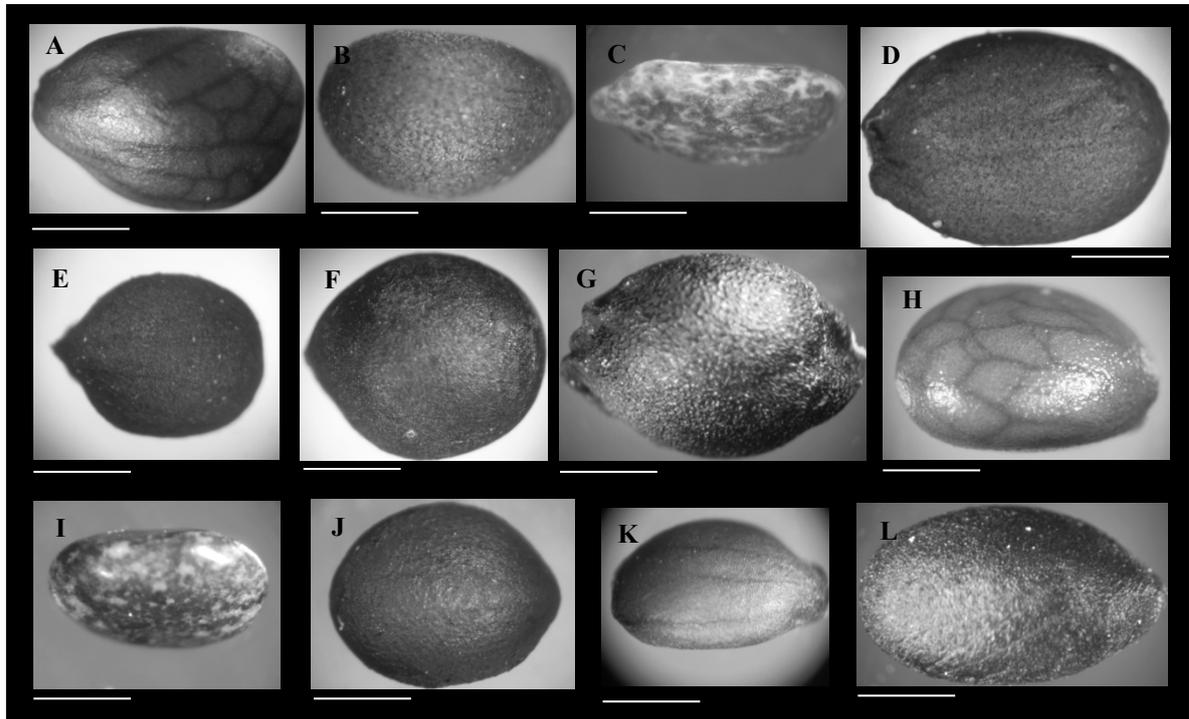
*reticulate*: with a reticulum or net and interspaces characterized by raised walls; the interspaces can be distinct and relatively large,

*verrucose*: covered with irregularly shaped lobes or wart-like protuberances.

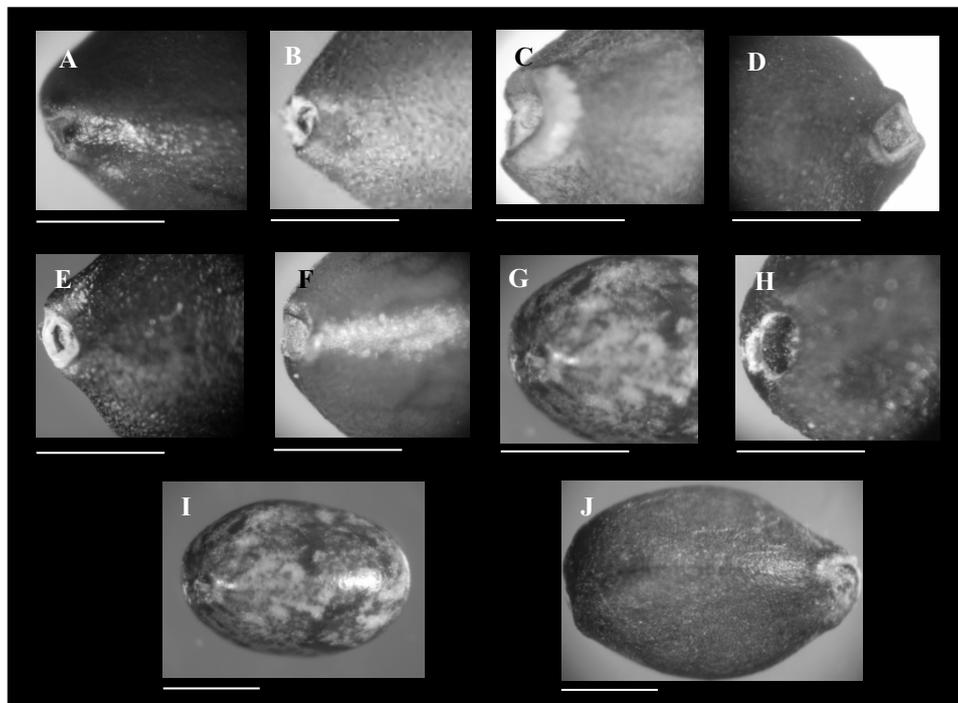
**Table 1: Morphological features of *Salvia* nutlets**

No.	Species	Nutlets shape	Nutlets length min-max value	Nutlets surface	Nutlets colour	Abscission scarshape	R (medium value)
1.	<i>S. aethiopis</i>	Ellipsoidal, triangular	2,1-2,7 mm	Colliculate	Brown with dark stripes	Triangular	9,5
2.	<i>S. austriaca</i>	Ovoid or globose, near triangular	1,9-2,5 mm	Verrucose	Light-brown	Circular	8
3.	<i>S. coccinea</i>	Ellipsoidal-elongated	2,1-2,6 mm	Reticulate	Brown marbled	Irregular	7
4.	<i>S. glutinosa</i>	Ovoid or ellipsoidal	2,9-3,8 mm	Colliculate	Brown with dark net	Irregular cercle	4,5
5.	<i>S. nemorosa</i>	Ovoid, triangular	1,4 -2,1mm	Verrucose	Dark brown with longitudinal stripes	Irregular	5
6.	<i>S. officinalis</i>	Irregular globose	2-3 mm	Colliculate	Dark brown	Irregular cercle	4
7.	<i>S. ringens</i>	Ovoid, near triangular	2,9-4 mm	Verrucose	Dark brown	Irregular	4
8.	<i>S. sclarea</i>	Ellipsoidal, triangular	1,9-3,1 mm	Foveolate	Brown with dark net	Irregular	5,5
9.	<i>S. tiliifolia</i>	Ellipsoidal, triangular	1,5-2 mm	Reticulate	Brown marbled	Circular	18,5
10.	<i>S. verbenaca</i>	Ovoid	1,8-2,7 mm	Colliculate	Dark brown with longitudinal stripes	Circular	4
11.	<i>S. verticillata</i>	Ellipsoidal, near triangular	1,5-2 mm	Verrucose	Brown with longitudinal stripes	Circular	3
12.	<i>S. viridis</i>	Ellipsoidal	2,4-3 mm	Colliculate	Brown	Circular	4,5

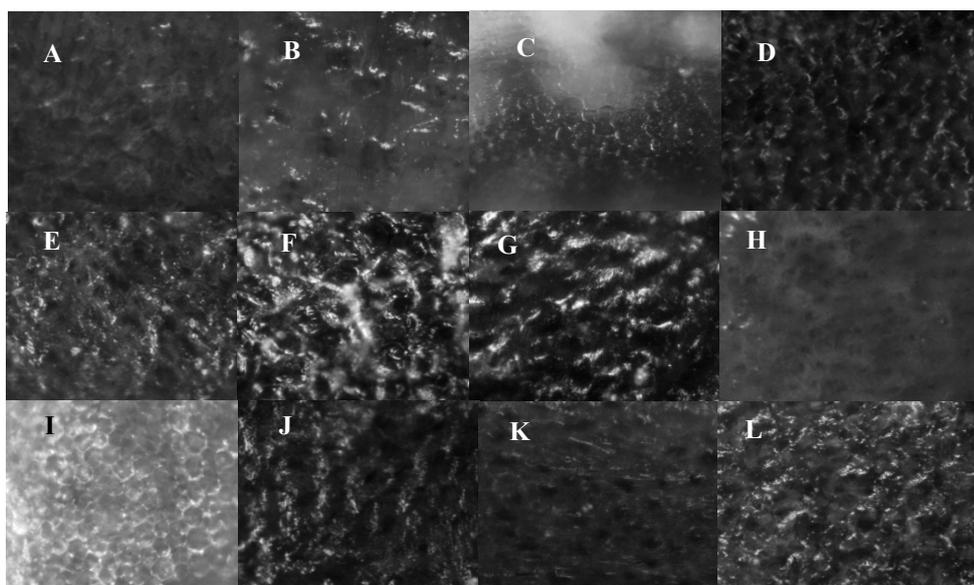
The production of mucilage by seeds or dried indehiscent fruits following their contact with water is a phenomenon present in many plant families, amongst them Lamiaceae. In this case, this phenomenon is called myxospermy although for the fruit the correct term is probably myxocarpy [19]. The study by Budantsev and Lobova in the tribe *Nepeteae* [1] shows that mucilage cells in the exocarp, when in contact with water, become elongated, destroy the cuticle, and take the shape of “mucilage hairs” or “papillae”.



**Fig. 1: Macroscopic aspect of nutlets - ventral surface.** A. *Salvia aethiopsis*, B. *S. austriaca*, C. *S. coccinea*, D. *Salvia glutinosa*, E. *S. nemorosa*, F. *S. officinalis*, G. *S. ringens*, H. *S. sclarea*, I. *S. tiliifolia*, J. *S. verbenaca*, K. *S. verticillata*, L. *S. viridis* (bars = 1000  $\mu$ m)

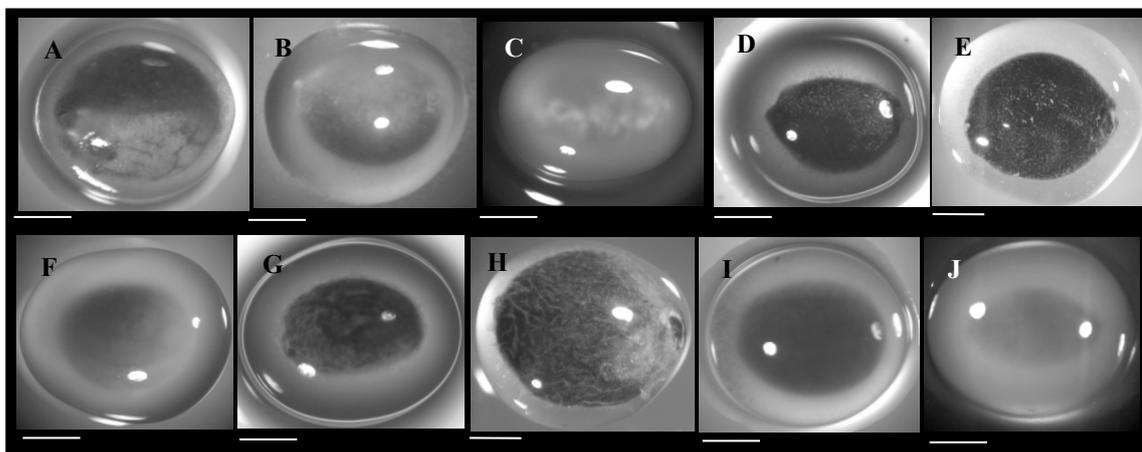


**Fig. 2: Abscission scar shape:** A. *Salvia aethiopsis*, B. *S. austriaca*, C. *Salvia glutinosa*, D. *S. nemorosa*, E. *S. ringens*, F. *S. sclarea*, G. *S. tiliifolia*, H. *S. verbenaca*. Differences between nutlets diameter and abscission scar diameter: I. *S. tiliifolia*, J. *S. verbenaca* (bars = 1000  $\mu$ m)



**Fig. 3: Micromorphological aspects of *Salvia* nutlets.** A. *Salvia aethiopsis*, B. *S. austriaca*, C. *S. coccinea*, D. *Salvia glutinosa*, E. *S. nemorosa*, F. *S. officinalis*, G. *S. ringens*, H. *S. sclarea*, I. *S. tiliifolia*, J. *S. verbenaca*, K. *S. verticillata*, L. *S. viridis* (x 700)

Our observations have shown that not all nutlets from the species studied exhibit this phenomenon. Thus, in *S. glutinosa* and *S. officinalis* the characteristic mucilage formation [1, 9, 19] was not observed. One notable aspect has been noted in *S. viridis*, a species in which the manifestation of myxocarpy (mucilage formation) was extremely short (about 2 minutes!)



**Fig. 4: Mucilage aspects:** A. *Salvia aethiopsis*, B. *S. austriaca*, C. *S. coccinea*, D. *S. nemorosa*, E. *S. ringens*, F. *S. sclarea*, G. *S. tiliifolia*, H. *S. verbenaca*, I. *S. verticillata*, J. *S. viridis* (bars = 1000  $\mu$ m)

The amount of mucilage produced by each species is different. The consistency of the mucilage also differs; in most species it is transparent, while only four of them form opaque mucilage (Fig. 4).

The characteristics of the myxocarpy phenomenon are presented in Table 2.

Table 2: Characteristic of myxocarpy

Species	Presence of myxocarpy	Mucilage consistency
<i>S. aethiopis</i>	+	transparent
<i>S. austriaca</i>	+	transparent
<i>S. coccinea</i>	+	opac
<i>S. glutinosa</i>	-	-
<i>S. nemorosa</i>	+	transparent
<i>S. officinalis</i>	-	-
<i>S. ringens</i>	+	transparent
<i>S. sclarea</i>	+	opac
<i>S. tiliifolia</i>	+	transparent
<i>S. verbenaca</i>	+	opac
<i>S. verticillata</i>	+	transparent
<i>S. viridis</i>	+	opac

### Discussion

Of the taxa studied here, the two American species of *Salvia* (*S. coccinea* and *S. tiliifolia*) are distinguished by their ellipsoidal or oval-ellipsoidal nutlets, brown-mottled, with a reticulate surface. In addition to these characteristics, they also have a relatively large ratio of the nutlet diameter to abscission scar diameter, 9 and 18.5 respectively.

The species of Eurasian and/or North African origin exhibit nutlets with a relatively uniform surface, colliculate or verrucose, except for *S. verbenaca*, a species with seeds displaying a foveolate surface. The more detailed study of nutlet surface shows obvious differences from one species to another [11, 12]. In *Flora R.P.R.* Guşuleac [7] described the nutlets from *S. aethiopis*, *S. glutinosa* and *S. verticillata* as smooth. Observations made at a higher magnification show that the surface has ornamentation described in the literature as colliculate (*S. aethiopis* and *S. glutinosa*) or verrucose (*S. verticillata*). The results of macro- and micro-morphological observations of nutlets suggest that they may present a taxonomically discriminating value for the 12 *Salvia* species studied.

In order to illustrate how the data cited above can be used in taxonomy, a identification key (based on nutlet characters) of the 12 species of *Salvia* has been performed (as an exercise).

Identification key of studied *Salvia* taxa depends on fruits characters:

1. Dark brown nutlets ..... 2
- 1'. Different coloured nutlets ..... 4
2. Nutlets showing myxocarpy ..... 3
- 2'. Nutlets not showing myxocarpy ..... *S. officinalis*
3. Transparent mucilage ..... *S. nemorosa*
- 3'. Opaque mucilage ..... *S. verbenaca*
4. Marbled nutlets ..... 5
- 4'. Nutlets not marbled ..... 6
5. Transparent mucilage ..... *S. tiliifolia*
- 5'. Opaque mucilage ..... *S. coccinea*
6. Nutlets not showing myxocarpy ..... *S. glutinosa*
- 6'. Nutlets showing myxocarpy ..... 7
7. Nutlets with an uneven apex ..... *S. ringens*

7'. Nutlets with a smooth apex .....	8
8. Nutlets with circular abscission scar .....	9
8'. Nutlets with abscission scar of shape other than circular .....	11
9. Verrucose nutlets .....	<i>S. verticillata</i>
9'. Colliculate nutlets .....	10
10. Nutlets with the nutlet/abscission scar ratio 7,5-9,5 .....	<i>S. austriaca</i>
10'. Nutlets with the nutlet/ abscission scar ratio 4,5-5 .....	<i>S. viridis</i>
11. Colliculate nutlets .....	<i>S. aethiopsis</i>
11'. Foveolate nutlets .....	<i>S. sclarea</i>

Production of mucilage, a phenomenon for which Hedge (1970) provided some of the most important information, can be considered an extra taxonomic character [9, 21]. The observations presented in this paper do not entirely confirm the conclusions of Ryding (2001) [20].

In his 1992 paper, Ryding suggests that plants that grow in dry habitats usually possess myxodiaspory [19], and therefore exhibit myxocarpy, while in his 2001 paper [20] he affirms that there is no obvious correlation between the occurrence of myxocarpy and the type of habitat (dry or humid) in which the plants grow.

The results of our studies confirm the latter view. That is to say the nutlets of the species *S. aethiopsis*, *S. austriaca*, *S. nemorosa*, *S. ringens*, *S. sclarea*, *S. verbenaca* and *S. verticillata* (xerophytes or xeromesophytes) produce mucilage when they are in contact with water, while those of the mesohydrophyte *S. glutinosa* and the xerophyte *S. officinalis* do not exhibit myxocarpy.

Regarding correlation between nutlet size and the formation of mucilage, both Hedge [9] and Ryding [20] noticed that species with large nutlets (over 2 mm<sup>3</sup> according to Ryding) rarely form mucilage while those with a reticulate surface never do. This hypothesis cannot be extrapolated to all species of the genus *Salvia*, as some such as *S. aethiopsis* or *S. ringens* have large nutlets, similar to *S. officinalis*, and exhibit myxocarpy; as does *S. verbenaca*, which has nutlets that are both large and have a reticulate surface.

It is likely that several factors are involved in this phenomenon, alongside nutlet shape or size, soil properties or even a genetic factor that requires further study.

### Conclusions

The study of the surface of the nutlets in *Salvia* highlights characters that may be useful to clarify some taxonomic aspects at species level. A rarely used index is represented by the ratio of the maximum diameter of the nutlet and that of the abscission scar. This ratio presents different values between species.

The phenomenon of myxocarpy requires further study to clarify the differences between species that are relatively similar in distribution, habitat or ecology.

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## MORFOLOGIA NUCULELOR UNOR SPECII DE SALVIA (LAMIACEAE)

### (Rezumat)

Genul *Salvia*, cel mai bine reprezentat în cadrul familiei *Lamiaceae*, cuprinde specii cu utilizări diferite: medicinale, decorative, melifere etc. În lucrarea de față ne propunem să aducem noi informații privind morfologia nuculelor de la 12 specii de *Salvia*. Materialul botanic a fost colectat din Grădina Botanică Iași sau din diferite locații din țară și este reprezentat de taxoni cultivați sau spontani.

Cu ajutorul lupei binocular și al microscopului fonic sunt evidențiate caracteristicile testei referitoare la culoare, dimensiuni, prezența și dispunerea cicatricei absciyonale, prezența ornamentațiilor. Fenomenul de mixocarpie, caracteristic familiei *Lamiaceae*, a evidențiat diferențe în ce privește prezența sau absența sa, viteza de manifestare, consistența mucilagiului caracteristic. Observațiile asupra acestui fenomen diferă de unele informații din literatura de specialitate, de aceea sunt necesare studii suplimentare.

Analiza sintetică a tuturor datelor obținute împreună cu iconografia detaliată a nuculelor completează informațiile existente în literatură și poate avea utilitate practică în clarificarea unor aspecte de taxonomie a genului *Salvia*.