

PALYNOLOGICAL FEATURES AND POLLEN GERMINATION AT *SCILLA BIFOLIA* L.

CARACTERISTICILE PALINOLOGICE ȘI GERMINAREA POLENULUI DE *SCILLA BIFOLIA* L.

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Abstract. *The paper presents the main features palinological of Scilla bifolia L. ssp. bifolia on the one hand and the potential germination of pollen same taxon, on the other hand. Palinological measurements focused: shape, color of pollen grains, exin ornamentation, size of pollen grains, the number of germination pore/grain of pollen. Testing of germinating potential was carried out by "in vitro" on nutrient mediums with varied concentration in carbohydrate elements between 0% and 100%. The pollen germination potential of Scilla bifolia is expressed at its peak when in culture medium is sucrose 15%, 20%, 25%, 24 hours after inoculation when the number of pollen grains germinated is more than 70%. The pollen germinated "in vitro" is viable over 144 hours. The results prove a normal meiosis and well balanced male gametes, so fertile for this diploid genotype. Investigations carried provides important information in taxonomy for genus Scilla and ornamental plant breeding.*

Key words: *Scilla bifolia*, pollen grain, nutritive medium, germination capacity

Rezumat. *În lucrare se prezintă principalele caracteristici palinologice la Scilla bifolia L. ssp. bifolia, pe de o parte, și potențialul germinativ al polenului aceluiași taxon, pe de altă parte. Determinările palinologice au vizat: forma, culoarea granulelor polinice, ornamentația exinei, dimensiunea granulelor de polen, numărul porilor germinativi/granulă de polen. Testarea potențialului germinativ s-a realizat prin germinări "in vitro" pe medii nutritive cu concentrație variată în elemente glucidice, cuprinse între 0% și 100%. Potențialul germinativ al polenului de Scilla bifolia se exprimă la cote maxime în condițiile prezenței în mediul de cultură a zaharozei în concentrații cuprinse între 15% și 25%, după 24 ore la inoculare, când numărul granulelor de polen germinate a depășit 70%. Polenul germinat "in vitro" este viabil peste 144 ore. Rezultatele dovedesc o meioză polinică normală și gameți masculi echilibrați, deci fertili la acest genotip diploid. Investigațiile efectuate furnizează informații cu importanță în taxonomia genului Scilla și ameliorarea plantelor ornamentale.*

Cuvinte cheie: *Scilla bifolia*, granule de polen, mediu nutritiv, potențial de germinare

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INTRODUCTION

Morphological variation in many features of the wall of pollen grains is a correlation between morphology and function of the pollen, correlation realized by natural selection (Matamoro-Vidal *et al.*, 2016).

The importance of the exine ornamentation for taxonomy is argued by Jamzad and Hasani-Nejad (2014) which have realized a study about the pollen of *Scutellaria* genus. Their results reveal that the exine ornamentation is a diagnostic character useful for the infrageneric classification of *Scutellaria*.

The pollination, pollen, fertilisation and seed formation have been the subject of research for to establish the self-regeneration capacity of the species (*Arbutus andrachne* and *Osmanthus decorus*) of wild flora that are included in the Red Data Book of the Georgian SSR (Melia *et al.*, 2012).

Scilla L. genus comprises about 100 species distributed in south Europe, the Mediterranean region, and central and western Asia. According to recent studies based on molecular data, *Scilla* genus is not monophyletic. For generic delimitation, an important role have the pollen characters that help to evaluate the taxonomic value (Ghavami *et al.*, 2009). *Scilla* L. genus is very heterogeneous by point of view genetically, which is reflected in increased variability of morpho-physiologic particularities. In Romania, *Scilla bifolia* is represented by two subspecies: *bifolia* and *drunensis* (Kunigunga, 2015). *S.b.* ssp. *bifolia* is diploid ($2n=2x=18$) and *S.b.* ssp. *drunensis* is tetraploid ($2n=4x=36$) (Greilhuber and Speta., 1977; Kochjarová *et al.*, 2004; Kochjarová, 2005; Mráz, 2007). This study aimed to determine the morphological and germination capacity of pollen from *Scilla bifolia* L. ssp. *bifolia*.

MATERIAL AND METHOD

The biological material is represented by the fresh pollen of *Scilla bifolia*. For to define the pollen morphology, was evaluated: shape of pollen grains, exine sculpturing, size of pollen grains and number of germinative pores/pollen grain. For determining the shape of pollen grains, the apertures, the exine sculpturing, we have used the Hund Wetzlar light microscope, at which we took microphotographs. For determining the size of pollen we did micromesurements at 1000 pollen grains. The values obtained were statistically processed, resulting the biostatistics indexes. For establishing the number of germinative pores/pollen grain, we have done determinations on 1000 pollen grains. The method consisted in introducing the pollens in a mixture of sulphuric acid and acetic acid.

For study of the germination pollen, we have used the hanging drop method (Stanley and Linskens, 1985). The nutritive mediums necessary for the germination of pollens consisted in distilled water, agar 1% and sucrose at different concentrations: 0%, 5%, 10%, 15%, 20%, 25%, 40%, 50%, 70% and 100%. Thus, 10 experimental variants resulted. For each experimental variant, we have used 10 "wet rooms". The amount of inoculated pollen per each medium was the same in all cases. Readings at the Hund Wetzlar optic microscope were done at 1.5, 24, 48, 72, 120, and 144 hours since the pollen inoculation in mediums, thus, being established the percent dynamics of the germination capacity for this genotype. The germination capacity was

expressed as percentage, by reporting the number of germinated grains to total pollen grains.

RESULTS AND DISCUSSIONS

The palynological characterization of *Scilla bifolia* L. ssp. *bifolia*

The pollen of *Scilla bifolia* is ellipsoidal, yellow colors (fig. 1). The surface sculpturing is reticulata (fig. 2). Each pollen grain has a single aperture which houses one germinative pore (fig. 2 – right). Because *Scilla bifolia* is a liliata, it is normal to have only one pollen aperture/grain pollen. Our results are generally congruent with the circumscription of the new genera previously included in *Scilla* and show that pollen exine morphology is useful as an additional taxonomic character for the delimitation these segregate genera. Our results are congruent with data from literature and show that pollen exine morphology is useful as an additional taxonomic character for the taxonomic delimitation of *Scilla* genus very heterogeneously (Ghavami *et al.*, 2009).

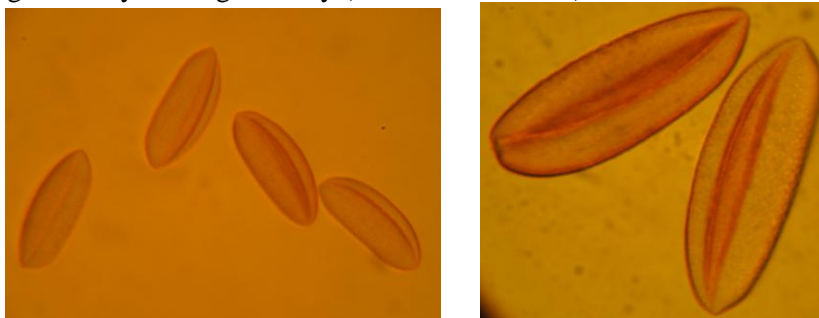


Fig. 1 The shape of pollen grains of *Scilla bifolia*: 400X(left); 1000X (right) (Original)

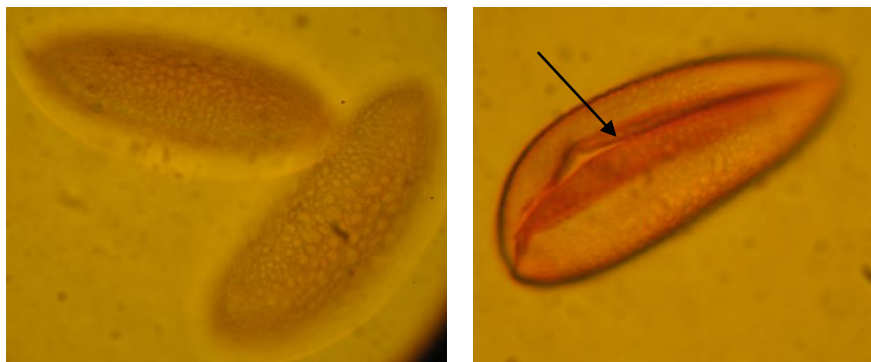


Fig. 2 The exine sculpturing of *Scilla bifolia* (left); Pollen grain to which is indicated the aperture (right) (1000X) (Original)

Concerning to size of pollen grains, the polar axis has an average of 62.88 μm and the equatorial axis is 25.46 μm . The ratio between the two diameters is 2.47. The coefficient of variation (s%) show low to middle variability for the polar axis and middle variability for equatorial axis (tab. 1).

Table 1

Variability of pollen grain size in *Scilla bifolia* ssp. *bifolia*

Type of axis	Mean value (μm)	Minimum value (μm)	Maximum value (μm)	Variation height (μm)	S (μm)	S%	$S - \bar{x}$ (μ)	Ratio P/E (μm)
Polar axis (P)	62.88	29.33	70.73	41.40	5.74	9.12	0.57	2.47
Equatorial axis (E)	25.46	17.25	34.50	17.25	2.73	10.73	0.27	

The pollen germination process of *Scilla bifolia* ssp. *bifolia*

The results concerning to pollen germination of *Scilla bifolia* are summarized in table 2.

Table 2

The germination capacity (%) of *Scilla bifolia* ssp. *bifolia*

Time of inoculation	% sucrose in medium									
	0%	5%	10%	15%	20%	25%	40%	50%	70%	100%
after 1.5 hours	28	29	29	0	0	0	0	0	0	0
after 24 hours	27	27	46	75	73	70	31	0	0	0
after 48 hours	12	22	51	81	80	75	43	40	33	17
after 72 hours	7	21	47	80	82	73	47	41	30	17
after 120 hours	0	20	43	78	85	71	48	43	25	15
after 144 hours	0	20	40	77	85	70	48	44	24	4

After 1.5 hours from inoculation of pollen on nutrient mediums, it was found that only the poorest mediums in sucrose (0%, 5%, 10%) have allowed early sprouting process. 24 hours after inoculation, pollen have germinated on yet four mediums variants. At this moment, the highest levels of pollen germinated it is recording on mediums with 15%, 20% and 25% sucrose (fig. 3).

After 48 hours from the inoculation, pollen have germinated on all ten variants of mediums. The proportion of germination varies depending on the concentration of sucrose in medium. The highest percentage values of germination are obtained on the mediums with 15%, 20% and 25% sucrose. After 72 hours from the inoculation, we find that on some mediums the percentage of pollen germinated decreases, on other mediums, these increases. Thus, on medium sucrose free, is a marked decrease in the level of germination. On the mediums enriched with 5%, 10%, 15%, 25% and 70% sucrose, the germination decreases slightly. On the mediums with 20%, 40% and 50% sucrose germination rates increases slightly. On the medium most concentrated in sucrose (100%), the rate germination remains unchanged. After 120 hours after inoculation, on the medium without sucrose, the pollen tubes are all broken. On the mediums with

20%, 40% and 50% sucrose are ongoing insignificant increases in germination rate. After 144 hours after inoculation, the situation is not much changed from the previous period. On mediums with 20% and 40% sucrose, germination rate remains the same. Only on medium with 50% sucrose has been a very slight increase of rate of germination. We mention that on medium with 100% sucrose it produces a sharp decrease of germination.

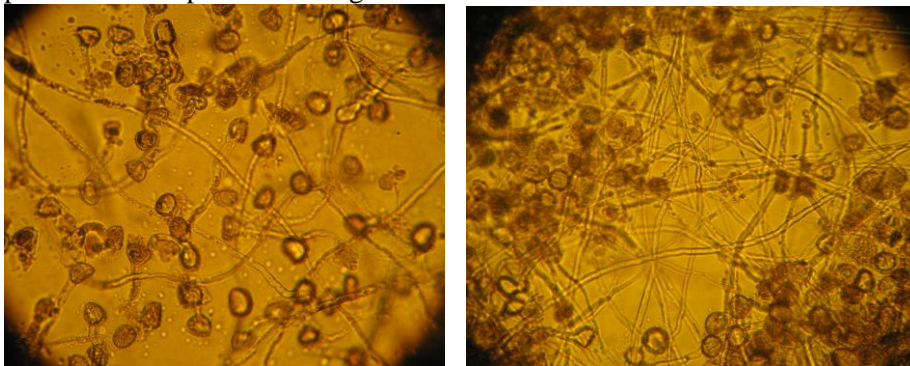


Fig. 3 Pollen germination 24 hour after inoculation in *Scilla bifolia*: on medium with 10% sucrose (left); on medium with 20% sucrose (right) (100X) (Original)

The dynamic analysis of pollen germination rate of *Scilla bifolia* (fig. 4) show that in the first 24 hours after inoculation it marks a significant jump of increase of germination capacity of pollen on three culture mediums with 15%, 20% and 25% sucrose. On these three mediums, pollen germination rates of *Scilla bifolia* was expressed at maximum levels, ranging between 70-75%.

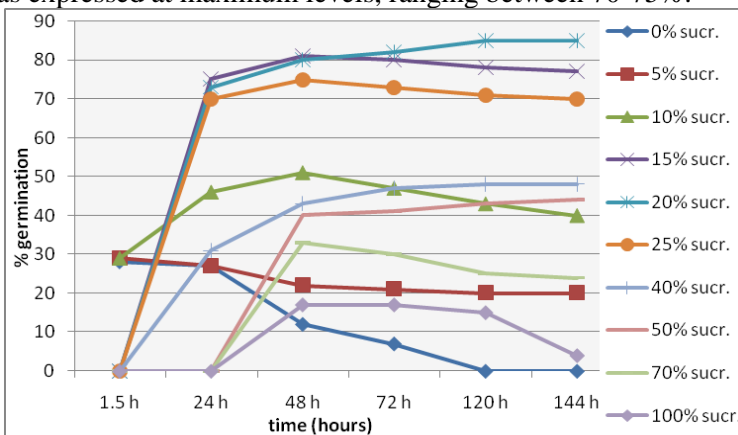


Fig. 4 The germination dynamics of pollen in *Scilla bifolia*

In following interval of times, also significant increases no longer produced on neither any medium variant. In the following hours, germination rates on the three favorable mediums were maintained in narrow ranges. Other types of medium which was probed germination capacity have proven to be ineffective for the pollen of *Scilla bifolia*. The hypotonic mediums (with 0%, 5% and 10% sucrose) and the hypertonic

mediums (with 40%, 50%, 70% and 100% sucrose) do not allow reaching the maximum capacity of pollen germination of this genotype.

CONCLUSIONS

1. Palynological determinations morphological (shape, color of pollen grains, exine ornamentation, the size of two axes pollen, number of germinative pores/pollen grain) are additions with taxonomic value for *Scilla bifolia* ssp. *bifolia*.

2. Determinations on the variability of pollen grains size indicate a high homogeneity, which is in close correlation with pollen germination of this genotype.

3. The germination capacity pretty high of pollen in *Scilla bifolia* is an argument that supports a normal polinic meiosis, so the male gametes genetically well balanced.

4. The pollen of *Scilla bifolia* manifest preference for nutritious substrates with 15%, 20% and 25% carbohydrate, substrates that provide high level expression of potential germination. This means that "in vivo" the pollens of *Scilla bifolia* germinate well in stigmatic liquid a native concentration in carbohydrate of 15-25% after approx. 24 hours after pollination.

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