PALYNOLOGICAL FEATURES AND POLLEN GERMINATION POTENTIAL IN *HYACINTHUS ORIENTALIS* L.

PARTICULARITĂŢILE PALINOLOGICE ŞI POTENŢIALUL GERMINATIV AL POLENULUI DE *HYACINTHUS ORIENTALIS* L.

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Abstract. *Palynology is a field of research on pollen and plant spores. Pollen research provides phylogenetic evidence in plant systematics. In this study are reported the main palynological features of a genotype known in the culture of ornamental plants, namely Hyacinthus orientalis. Respective palynological determinations concerned: shape, color of pollen grains, ornamentation of exine, size of pollen grains, number of germination pores/pollen grain. In connection with these determinations, the germination potential was tested on nutrient mediums with different carbohydrate compositions. In this regard, the effects of sucrose and glucose in different concentrations on pollen germination rates were investigated. Hyacinthus orientalis pollen has been shown to prefer 15% sucrose, when percentage of germinated grains was 95%, a percentage that is maintained for the next 96 hours. Glucose were suboptimal for pollen of this genotype. The results of this experiment provide useful information in taxonomy of genus Hyacinthus and in ornamental plant breeding.*
Key words: *Hyacinthus orientalis*, pollen grain, nutritive medium, pollen germination

Rezumat. *Palinologia este domeniul de cercetare al polenului şi sporilor plantelor. Cercetările asupra polenului furnizează dovezi filogenetice în sistematica plantelor. În prezentul studiu sunt relatate principalele particularităţi palinologice ale unui genotip cunoscut în cultura plantelor ornamentale, şi anume Hyacinthus orientalis. Respectivele determinări palinologice au vizat: forma, culoarea granulelor polinice, ornamentaţia exinei, dimensiunea granulelor de polen, numărul porilor germinativi/granulă de polen. Corelat cu aceste determinări s-a procedat la testarea potenţialului germinativ pe medii nutritive cu compoziţii glucidice diferite. În acest sens au fost investigate efectele zaharozei şi ale glucozei în diferite concentraţii asupra ratelor de germinare a polenului. S-a demonstrat că polenul de Hyacinthus orientalis preferă medii nutritive îmbogăţite cu 15% zaharoză, când procentul granulelor germinate a fost de 95%, procent care se menţine şi în următoarele 96 ore. Glucoza a fost suboptimală pentru polenul acestui genotiop. Rezultatele prezentului experiment oferă informaţii utile în taxonomia genului Hyacinthus şi în ameliorarea plantelor ornamentale.*
Cuvinte cheie: *Hyacinthus orientalis*, granulde polen, mediu nutritiv, germinarea polenului

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INTRODUCTION

Palynology is the study of pollen and spores. Pollen grains and spores have certain features, which provide important information for inferring the phylogenetic relationships of plants. Also, the features of pollen grains and spores help identify a particular plant taxon. Furthermore, these features it has been shown to have great benefit to the criminal investigator (Adams-Groom B., 2012; Adams-Groom B., 2015). Pollen grains are unique for each vegetable taxon, having a certain shape, size, ornamentation of the exine (Erdman, 1952).

Hyacinthus is a monocot genus of bulbous plants, native to the eastern Mediterranean. *Hyacinthus orientalis* L. is among the few species of this genus, is rare in the wild but common in the cultured landscape. This species has not only decorative value, but is also an important source of pollen and nectar for supporting valuable pollinators, such as *Apis mellifera* and *Bombus* spp. in early spring (Bożek , 2019).

Researchs about *Hyacinthus orientalis* pollen focused on the synthesis of RNA and proteins in vegetative and generative cells during the maturation of pollen grains, demonstrating that the rhythm of these syntheses is more accentuated in vegetative cell (Bednarska, 1984). Zienkiewicz K. *et al*. (2008a) deepen this researchs and confirms that transcriptional activity is more intense in vegetative cells in young pollen grains of *Hyacinthus orientalis* and decreases in mature pollen grains, reaching a minimum before anthesis. Using labeled antigens it has been shown that organization of splicing machinery elements during *Hyacinthus orientalis* pollen grain development undergoes spatial changes in the two pollen cells (Zienkiewicz *et al*., 2008b).

The present study aims to clarify the main palynological features and the degree of fertility in *Hyacinthus orientalis* pollen.

MATERIAL AND METHOD

The biological material was represented by the fresh pollen of *Hyacinthus orientalis.* For to determine the pollen features palynological, 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 Oxion light microscope, at which we took microphotographs. For determining the size of pollen we did micromeasurements 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 was based on introducing the pollens in a mixture of sulphuric acid and acetic acid.

The germination capacity of pollen was determined used the hanging drop method (Stanley and Linskens,1985). The nutrient mediums were prepared from distilled water in which two types of carbohydrates were dissolved: sucrose and glucose, in different concentrations. The type of carbohydrate and its concentration in distilled water contributed to preparing 12 experimental variants of mediums: sucrose enriched mediums: 5%, 15%, 25%, 50%, 70%, 100%; glucose enriched mediums: 5%, 15%, 25%, 50%, 70%, 100%. Along with the 12 variants of mediums with added

carbohydrates, a variant of medium without carbohydrates was prepared, label 0%. For each experimental variant, we have used 8 "wet rooms". The amount of inoculated pollen per each medium drop was the same in all cases. Readings at the Oxion optic microscope were done at 2, 24, 48, 72, 96 and 120 hours since the pollen inoculation in mediums, thus being established the percent dynamics of the germination capacity. The germination capacity was expressed as percentage, by reporting the number of germinated grains to total pollen grains.

RESULTS AND DISCUSSIONS

The palynological features of *Hyacinthus orientalis* L.

The pollen of is *Hyacinthus orientalis* elliptical, pale yellow color (fig. 1-left). The exine is reticulata (fig. 1- right).

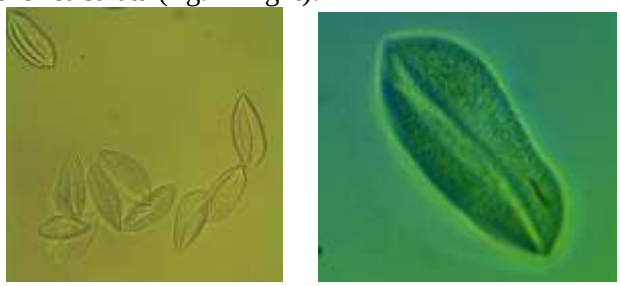

Fig. 1 The shape of pollen grains and the exine sculpturing of *Hyacinthus orientalis*: 400X(left); 1000X (right) (Original)

The pollen of *Hyacinthus orientalis* is monoporate, namely each pollen grain has only one germinative pore sheltered by an a single aperture (fig. 2). The presence of only one germinative pore/pollen grain is characteristic for all liliopsids species.

Fig. 2 Pollen grain to which is indicated the only germinative pore (1000X) (Original)

These palynological data about *Hyacinthus orientalis* pollen are in accord to SEM pollen images selected of British flora (https://www.worldplants.ca/documents/books/Pollen%20Project_web_Part2.pdf).

The size of pollen grains was estimated by measuring the two pollen axes. The polar axis has an average of 71.64 μm and the equatorial axis is 33.19 μm. The ratio between the two polinic axes is 2.16 μm. The coefficient of variation (s%) indicates low variability for both axes (tab. 1).

Table 1

Variability of pollen grain size in *Hyacinthus orientalis*

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)	71.64	51.75	82.80	31.05	4.99	6.97	0.69	2.16
Equatorial axis (E)	33.19	24.15	41.40	17.25	3.37	10.2	1.02	

The germination capacity of *Hyacinthus orientalis* pollen

The germination capacity of hyacinth pollen was tested on two categories of nutrients mediums which differs by the type of carbohydrate as mentioned in the section "Material and Method".

After 2 hours from inoculation of pollen on mediums, pollen germinated in high proportions on mediums with 15% carbohydrates, namely: 72% germinated pollen grains on sucrose and 50% on glucose. On mediums with 25% carbohydrates were registered: 20% germinated pollen grains on sucrose and 15% on glucose. On carbohydrate deficient mediums (0%, 5%), germination was very low. Mediums with 50-100% carbohydrates have not yet allowed the germination process to start (figs. 3 and 4).

After 24 hours from inoculation, germination percentages of pollen increased considerably, especially on mediums with 15% carbohydrates (95% on sucrose, respectively 61% on glucose). Among the glucose averages, the one with 25% offered higher chances (85%) for hyacinth pollen germination. The pollen also germinated on mediums with 50% and 70% carbohydrates, but at lower levels compared to the mediums with 15% and 25% carbohydrates. On low-carbohydrate mediums, germination rates remain low (3%), while on the most carbohydrate-rich mediums (100%), germination has not yet begun (figs. 3 and 4).

48 hours after pollen inoculation, we constate that are increases in germination rates on mediums with 15-70% carbohydrates. In all cases, germination on sucrose was higher than on glucose. Even on medium with 100% sucrose, 10% germinated pollen was registered, but not on glucose (figs. 3 and 4).

After 72 hours from inoculation, germination rates increase especially on mediums with 50-70% carbohydrates (figs. 3 and 4).

After 96 and 120 hours from inoculation, germination rates remain approximately constant compared to the previous interval, with specification that on mediums with 70%, germination rates decreases slightly (figs. 3 and 4).

The dynamics of germination during the 120 hours of analysis, show that in the first 24 hours after pollen inoculation occurs a significant increase in germination rate on most mediums variants (figs. 3 and 4).

In case of sucrose mediums, the highest levels of pollen germination were supported by variants of the mediums with 15% and 25%, when the percentage values reached 98% and 90%, respectively. The germination capacity high of

pollen in *Hyacinthus orientalis* is a proof that polinic meiosis at this genotype is normale and the male gametes are genetically well balanced.

In case of glucose mediums, profile of germination dynamics in the same time interval is somewhat irregular, however it indicates that the best of these is the with 15% glucose, which insured about 90% germinated pollen.

Hyacinth'pollen does not germinate optimally on hypotonic mediums (0%, 5% carbohydrates), nor on hypertonic mediums (50% - 100% carbohydrates). It should be noted that on medium with 100% glucose, pollen of this genotype did not germinate at all.

From these determinations, in addition to the information already presented, we extract another one, namely that the hyacinth pollen is viable for at least 120 hours. Research on the vitality of pollen in certain varieties of *Hyacinthus orientalis* shows that this one it is lost after 9 days of storage at room temperature, while storage for 12 days between 4 ^{0}C and -20 ^{0}C ensures a germination of over 20% (Li *et al.*, 2010).

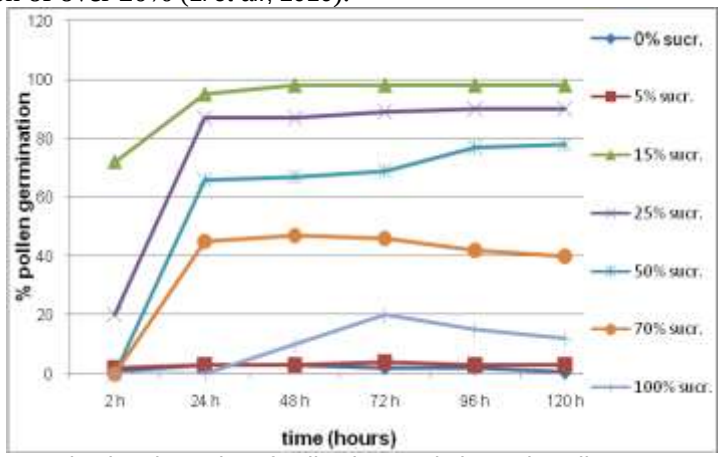

Fig. 3 The germination dynamics of pollen in *Hyacinthus orientalis* on sucrose mediums

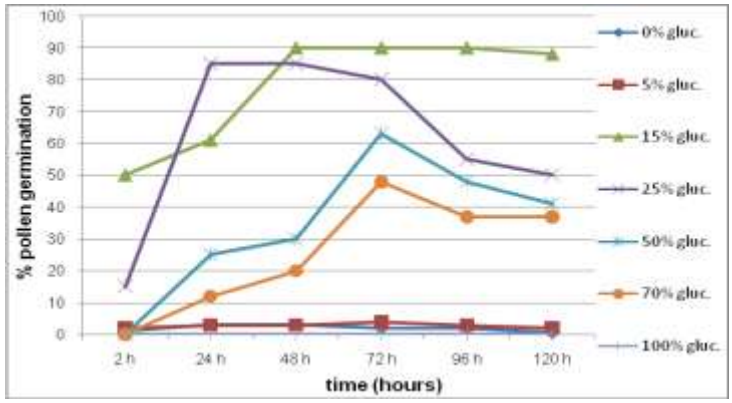

Fig. 4 The germination dynamics of pollen in *Hyacinthus orientalis* on glucose mediums

CONCLUSIONS

1. Palinological features for *Hyacinthus orientalis* comply with those reported in the literature.

2. Size of *Hyacinthus orientalis* pollen grains have one smalle variability, which means that it is homogeneous. This characteristic is closely correlated in positive sense, with the germination capacity of pollen.

3. *Hyacinthus orientalis* pollen germinates at maximum level (98%) if the germination medium is enriched with sucrose in a concentration of 15%, at most 25%.

4. Sucrose is preferred to glucose for the optimal development of the male hyacinth gametophyte. This aspect proves that sucrose is more energy efficient than glucose, ensuring the optimal energy for hyacinth pollen in the germination process.

5. Our investigations have demonstrate that *Hyacinthus orientalis* pollen has a viability of at least 5 days (120 hours) at room temperature. This aspect can be taken into account for hybridization works in order to create new varieties within this species.

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