PALYNOLOGICAL CHARACTERIZATION AND GERMINATION OF *TULIPA GESNERIANA* L. POLLEN

CARACTERIZAREA PALINOLOGICĂ ŞI GERMINABILITATEA POLENULUI DE *TULIPA GESNERIANA* L.

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Abstract: *In this paper are highlighted the main palynological characteristics of a popular genotype in the culture of ornamental plants, namely Tulipa gesneriana. Palynological investigations focused on: shape, color of pollen granules, exine ornamentation, size of pollen granules, number of germinative pores/pollen granule. The study is completed by establishing the germination capacity of pollen grains, for which tests of germination capacity were performed on carbohydrate-enriched artificial mediums in different concentrations. Carbohydrates added to the nutrient mediums were represented by sucrose and glucose, depending on which two categories of nutrient media were formed: sucrose mediums and glucose mediums. Tulipa gesneriana pollen has been found to have a predilection for sucrose-containing nutrients compared to glucose. The highest level of germination (90%) was achieved on the medium with a concentration of 5% sucrose. The results of this study are important for the taxonomy of this genotype and in breeding programs.*
Key words: *Tulipa gesneriana*, pollen grain, sucrose, glucose, pollen germination capacity

Rezumat. *În prezenta lucrare sunt evidenţiate principalele caracteristici palinologice ale unui genotip popular în cultura plantelor ornamentale şi anume Tulipa gesneriana. Investigaţiile palinologice au vizat: forma, culoarea granulelor polinice, ornamentaţia exinei, dimensiunea granulelor de polen, numărul porilor germinativi/granulă de polen. Studiul este completat de stabilirea capacităţii de germinare a granulelor de polen, pentru care s-au realizat testări ale capacităţii de germinare pe medii artificiale îmbogăţite cu glucide în diferite concentraţii. Glucidele adăugate în mediile nutritive au fost reprezentate de zaharoză şi glucoză, funcţie de care s-au constituit două categorii de medii nutritive: medii cu zaharoză şi medii cu glucoză. S-a constatat că polenul de Tulipa gesneriana are predilecţie pentru mediile nutritive cu adaos de zaharoză în raport cu cele cu glucoză. Cel mai ridicat nivel de germinare (90%) s-a realizat pe mediul cu concentraţia de 5% zaharoză. Rezultatele prezentului studiu au importanţă pentru taxonomia acestui genotip şi în programele de ameliorare.*
Cuvinte cheie: *Tulipa gesneriana*, granul de polen, zaharoză, glucoză, capacitatea de germinare a polenului

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INTRODUCTION

Pollen has been and is an very important research objective in plant biology. More and more recent studies using both light microscopy and scanning electron microscopy, highlight that the pollen morphological features investigations (palynological characters) have an unparalleled contribution in the precise identification and delimitation of species, families, subfamilies, genres, tribes etc. (Cui *et al*., 2018; Cui *et al*., 2020; Hu *et al*., 2020; Souza *et al*., 2021; Ullah *et al*., 2019; Umber *et al*., 2021; Wrońska-Pilarek *et al.*, 2011). In situations where intrageneric relationships are controversial and hard to delimit, morphological palynological details establish the phylogenetic relationships between those plant species (Hu *et al*., 2020). Some studies have established based on the morphology of pollen has an important significance, based on which differentiate salt-tolerant plant species (Nazish *et al*., 2019). Palynology studies also have their applicability in beekeeping, in which case they are known as melissopalynological studies and aim to identify the botanical origin of honey (Pospiech *et al*., 2021).

In the present study, *Tulipa gesneriana* pollen was studied. Although this genotype is most often propagated by bulbs, there is always an intense concern of horticulturists to create new varieties of tulips, as diverse as possible. In this sense, pollen acquires a special importance in the directed hybridization works.

The directions of tulip pollen research in the present study were: the main palynological characteristics and the degree of fertility investigated by its germination capacity.

MATERIAL AND METHOD

The biological material was fresh pollen taken from 20 flowers of *Tulipa gesneriana* L. belonging to a simple perigon variety. The pollen features palynological was: shape of pollen grains, exine sculpturing, size of pollen grains, number of germinative pores/pollen grain.

The evaluation of the shape of pollen grains and the ornamentation of pollen grain exine was performed by observations and microphotographs under the Oxion light microscope, using the 10x eyepiece and the 100x objective.

The size of the pollen granules was determined by micro-measurements of a sample of 1000 pollen grains.The values obtained were statistically processed, resulting the biostatistics indexes.

Establishing the number of germinative pores/pollen grain was determined by applying the pollen dive method in a mixture of sulfuric acid and acetic acid. The effects of acids on pollen grains were monitored for a sample of 1000 pollen grains.

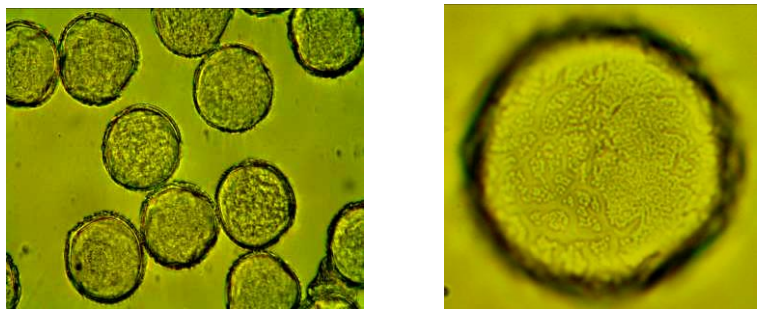
The germination capacity of pollen was determined used the hanging drop method. The nutrient mediums were prepared from distilled water in which two types of carbohydrates were dissolved: sucrose and glucose, in different concentrations. The type of carbohydrate and its concentration in distilled water 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, marked 0%. For each experimental

variant, we have used 10 “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. At each time interval, 2 microscopic fields/“wet rooms”/experimental variant were read, subsequently calculating arithmetic mean of readings. The germination capacity of the pollen was expressed as a percentage, by reporting the germinated grains to the total number of grains in the optical field.

RESULTS AND DISCUSSIONS

Palynological characterization of *Tulipa gesneriana* L.

The pollen of *Tulipa gesneriana* is spheroidal, lemon yellow color (fig. 1-A). The exine is finely reticulated (fig. 1-B).



A B

Fig. 1. Pollen grain of *Tulipa gesneriana*: A- the shape of pollen grains (400X); B - the exine sculpturing (1000X) (Original)

Analysis of the pollen sample proves that the pollen of *Tulipa gesneriana* is monoporous, in sense that each pollen grain is provided with a single germintive pore located in an aperture (fig. 2). The existence of only one germinative pore/pollen grain is characteristic of all liliopside species, of which *Tulip gesneriana* is part.

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

To estimate the size of pollen grains, a single axis was measured, namely the equatorial axis, because the shape of this pollen is spheroidal.

The average value of tulip pollen size is 68.38 μm, and the coefficient of variability (s%) is 7.54%, which indicates that the pollen of this genotype is uniform in terms of size (tab. 1).

Table 1

The pollen grain size in *Tulipa gesneriana*

Type of axis	Mean value (μm)	Minimum value (μm)	Maximum value (μm)	Variati-on height (μm)	S (μm)	S%	$s_{\bar{x}}$ (μ)
Equatorial axis (E)	68.38	55.20	79.35	24.15	5.15	7.54	0.75

The germination capacity of *Tulipa gesneriana* pollen

After the first 2 hours from inoculation of pollen on nutritive mediums it is found that germination started on three mediums variants, namely: medium without carbohydrates (0%), mediums 5% and 15% carbohydrates. Germination of tulip pollen on these mediums differs depending on type of carbohydrate. Thus, medium 5% sucrose allowed a germinability of 50%, while on medium 5% glucose, germination was 36%. On a 15% carbohydrate in mediums, germination was similar for sucrose and glucose (figs. 3 and 4).

24 hours after inoculation, tulip pollen germinates explosively. On all mediums variants (5% -100% carbohydrates), pollen germinated in different proportions, except for medium with 100% glucose. The highest proportion of germinated pollen was 90% on medium 5% sucrose. On medium 5% glucose, the germination was 79%. Quite high values of germination capacity were also achieved on mediums enriched with 15% carbohydrates, namely: 73% on sucrose and 62% on glucose. On mediums with concentrations higher than 15% carbohydrates, germination capacity gradually decreases (figs. 3 and 4).

After 48 hours from inoculation, on medium 5% sucrose, proportion of germinated pollen is maintained at the level of previous time interval. On all other mediums, germination capacity decreases, the more so as the nutrient medium is more concentrated in carbohydrates. Germination occurs on substrate enriched with 100% glucose, but only 2% (figs. 3 and 4).

After 72 hours from inoculation, decreasing values of pollen germination are recorded in all experimental variants. In case of mediums with 70% and 100% glucose, the germination rate is reduced to 0%. The medium without carbohydrates can no longer support pollen germination (figs. 3 and 4).

After 96 hours from inoculation, germination rates decrease even more in all experimental cases germination (figs. 3 and 4).

After 120 hours after inoculation, germination capacity continued to decrease, especially on glucose mediums. Moreover, on medium with 50% glucose, germination percentage is reduced to 0% (figs. 3 and 4).

The dynamic analysis of pollen germination capacity during 120 hours of monitoring highlights the fact that in the first 24 hours after pollen sowing,

germination energy is the strongest and manifests itself at its maximum potential on 5% carbohydrate mediums Tulip pollen has been shown to germinate optimally on 5% sucrose.

During the120 hours of monitoring, it was found that germination process was supported by a small number of mediums variants with glucose in compared to mediums with sucrose. The glucose mediums that supported germination were: after 72 and 96 hours were 4; after 120 hours were 3. Number of mediums with sucrose that sustained germination after 72 hours, 96 hours and 120 hours were each time 6 (figs. 3 and 4). Preference of tulip pollen for sucrose is in agreement with Yu et al. (2012).

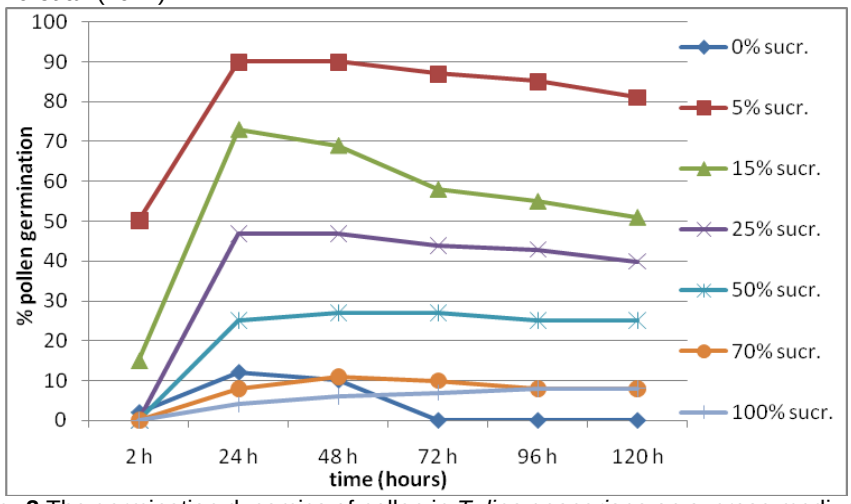


Fig. 3 The germination dynamics of pollen in *Tulipa gesneriana* on sucrose mediums

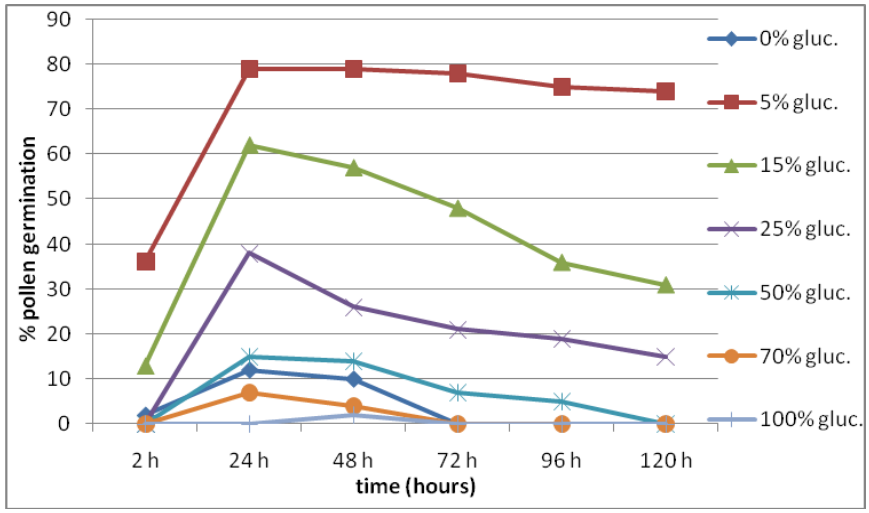


Fig. 4 The germination dynamics of pollen in *Tulipa gesneriana* on glucose mediums

CONCLUSIONS

1. Pollen of *Tulipa gesneriana* has relatively large dimensions and is uniform in size, as evidenced by coefficient of variability with a value below 10. Each tulip pollen bean has a single germinal pore, common to all liliopsides.

2. The maximum proven germination capacity of tulip pollen is 90%, achieved after 24 hours from seeding the pollen.

3. The germination process of tulip pollen is optimally performed on a hypotonic nutrient medium, with only 5% sucrose.

4. Tulip pollen has been shown to prefer sucrose to germination, not glucose, which has been shown to be suboptimal. This shows that sucrose is more energy efficient than glucose for germination process of this genotype.

5. Monitoring the germination capacity for 120 hours at 18 °C proved that tulip pollen has a viability of at least 5 days, especially on sucrose medium. This notification may provide important information for hybridization work to obtain new tulip varieties.

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