PECULIARITIES OF GROWTH OF POLLEN TUBE OF *TULIPA GESNERIANA* L.

PARTICULARITĂȚI ALE CREȘTERII TUBULUI POLINIC LA *TULIPA GESNERIANA* L.

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Abstract. *Building and growing the pollen tube are essential aspects of pollen biology. Pollen tube is a strategy for fertilizing siphonogamous plants by delivering immobile male gametes to the oosphere and central cell of embryonic sac, so that male gametes do not come into contact with external environment. The optimal length of the pollen tube is correlated with length of pistil that must pass through to achieve fertilization. The present study highlights the building and growth of tulip pollen tube in different environmental conditions. Environmental conditions differed in type and concentration of carbohydrates in the nutrient mediums. Two categories of mediums were created: with sucrose and with glucose. Tulip pollen was monitored for 120 hours. It has been shown that 5% sucrose in medium is more efficient than glucose, ensuring growth of the longest pollen tubes reaching 10000 µm. Research has further demonstrated viability of pollen tubes for 120 hours.*
Key words: *Tulipa gesneriana* , sucrose, glucose, pollen tube growth

Rezumat. *Edificarea şi creşterea tubului polinic sunt aspecte esenţiale ale biologiei polenului. Tubul polinic este o strategie de fertilizare a plantelor sifonogame prin livrarea gameţilor masculini imobili către oosferă şi celula centrală a sacului embrionar, astfel încât gameţii masculini nu intră în contact cu mediul extern. Lungimea optimă a tubului polinic este în corelaţie cu lungimea pistilului pe care trebuie să îl străbată pentru a realiza fertilizarea. Studiul prezent evidenţiază edificarea şi creşterea tubului polinic de lalea în condiţii diferite de mediu. Condiţiile de mediu au diferit prin tipul şi concentraţia carbohidraţilor din mediile nutritive. S-au realizat două două categorii de medii: cu zaharoză şi cu glucoză. Polenul de lalea a fost monitorizat 120 de ore. S-a demonstrat că zaharoza 5% în mediu este mai eficientă decât glucoza, asigurând creşterea celor mai lungi tuburi polinice care ating 10000 µm. Cercetările au demonstrat în plus viabilitatea tuburilor polinice timp de 120 ore.*
Cuvinte cheie: *Tulipa gesneriana,* zaharoză, glucoză, creşterea tubului polinic

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INTRODUCTION

The pollen tube is an extremely important acquisition in the evolution of plants. The short, slow-growing ancestral pollen tube became over time grew rapidly, very effective in administering male gametes to the egg cell. This evolutionary leap was made possible by the participation of the callus plugs, by involving specific enzymes encoded by orthologous genes (Abercrombie *et al.,* 2011). The pollen tube being a cell that grows in length serves as a real model for studying the influence of mechanical stimuli at the cellular level (Burri *et al*., 2018; Ghambari *et al*., 2018; Reimann *et al*, 2020). Also due to the fact that the pollen tube is a single cell, it serves as a model for quantitative micromechanical characterization, of colossal importance for the study of cell growth, cell-cell interaction. In this sense, a three-dimensional study method has been innovated (Läubli *et al*., 2021). Pollen tube studies on cellular stress due to mechanical stimuli can be reconfigured to characterize other peak-growing cells, such as roots, mycelial hyphae, moss protonemata (Burri *et al.*, 2018). The growth of the pollen tube is directly conditioned by a considerable supply of energy which is ensured by mitochondrial respiration but also by plastidial glycolysis (Selinski and Scheibeal, 2014). In the evolution of the pollen tube, an important aspect is its growth rate, considering that in the same style there is a competition between several pollen tubes that have the same purpose: reaching the oosphere and central cell of the embryonic sac to deliver male gametes (Parton *et al*., 2001; Williams *et al.*, 2016).

This study focuses on the peculiarities of growing *Tulipa gesneriana* pollen tube within 5 days under the influence of different carbohydrates in different concentrations in nutrient substrates, while highlighting the viability of pollen tube of this genotype.

The importance of this research is theoretical in that it contributes to the enrichment of knowledge on sexual reproduction of this genotype, but also practice by the fact that it is involved in directed pollination in order to obtain new varieties of tulip.

MATERIAL AND METHOD

The biological material is represented by the fresh pollen of *Tulipa gesneriana* L. For to determine the growth in dynamics of pollen tube, we have used the hanging drop method. 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 experimental 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. In parallel with determining germination capacity of pollen, were made micromeasurements for determining the

dynamics for extension of pollen tubes. In this respect, the readings at microscope were done at 2, 24, 48, 72, 96 and 120 hours since the pollen inoculation on nutritive mediums. The data shown represent arithmetic averages obtained from 2 microscopic fields/"wet rooms"/experimental variant.

Micromeasurements of pollen tube length were recorded directly by an ocular micrometer fitted to the eyepiece on microscope based on micrometer scale (μm). The length of the pollen tube was expressed by micrometers (μm). For pointing out the characteristics of pollen tubes from this genotype, photographs were taken at the Oxion light microscope.

To determine the length of floral ovary and of stamina it was calculated an average by measuring of 100 ovary and 600 stamens from 100 flowers of *Tulipa gesneriana* using the binocular magnifying glass Bel photonics.

RESULTS AND DISCUSSIONS

Two hours after inoculation of pollen on nutritive mediums, pollen tubes have already appeared on three variants of mediums, namely: 0%, 5% and 15% carbohydrates. The longest pollen tubes at this time have a length 394 μm, they being on medium with 5% sucrose (fig. 1, fig. 2).

After 24 hours from inoculation, an exponential increase in the length of pollen tubes is achieved on both sucrose and glucose mediums. After this time it is found that pollen tubes appeared on all mediums of sucrose and on mediums with 25-70% glucose. Thus, the longest pollen tubes grown on sucrose medium measure on average 9800 μm, and those on glucose medium have 4462 μm. It should be noted that mediums with 5% carbohydrates are those that support the highest growths of tulip pollen tubes (fig. 1, fig. 2).

After 48 hours, pollen tubes are constantly growing, recording the highest values on mediums with 5% carbohydrates. Thus, the tubes reach 10850 μm on 5% sugar and 6002 μm on 5% glucose. After this time from inoculation, pollen tubes appeared on medium with 100% glucose (fig. 1, fig. 2).

72 hours after pollen sowing, pollen tubes are still viable on most nutrient variants. The medium without carbohydrates (0%) as well as the mediums with glucose 70% and 100% no longer support the growth of pollen tubes. In case of sucrose mediums, there are insignificant increases on 15%, 70% and 100% concentrations. On sucrose 5%, pollen tubes are maintained at the values of the previous interval. In case of mediums with glucose, there are insignificant increases on 5% and 15% concentrations. Nutritive mediums with 70% and 100% glucose can no longer support presence of any pollen tubes. On all variants of mediums, partial degeneration of pollen tubes was observed by breaking them at tip (fig. 1, fig. 2).

After 96 hours from pollen sowing, it is found that on mediums enriched with 5-50% carbohydrates, pollen tubes have slightly lower values compared to previous interval. And on mediums with sucrose 70% and 100%, growth of pollen tubes continues (fig. 1, fig. 2).

After 120 hours of monitoring pollen tubes, their viability is noticeable, especially on mediums with 5% carbohydrates, even if there are slight decreases in values of their length. Of the two variant mediums, the one with sucrose 5% supports the longest pollen tubes (10700 µm) in whole experiment. Of the two variant mediums, the one with sucrose 5% supports the longest pollen tubes (10700 µm) in whole experiment. In case of mediums with sucrose, there are insignificant increases on concentrations 15%, 70% and 100%. On mediums with 15-50% sucrose and 15-25% glucose there are decreases in values of the length of the tubes. Mediums with 50% glucose no longer supports the existence of pollen tubes. Insignificant increases in pollen tube length occur only on mediums with 70% and 100% sucrose (fig. 1, fig. 2).

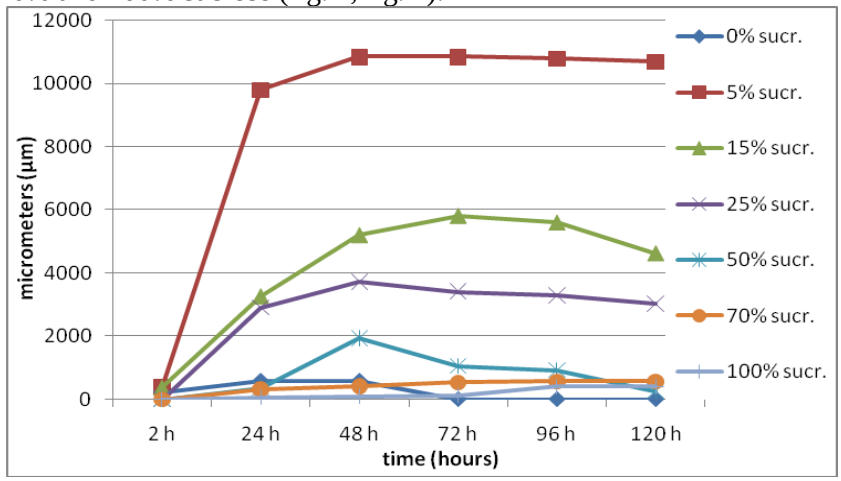


Fig. 1 Dynamics of average length of the pollen tube in *Tulipa gesneriana* on sucrose mediums

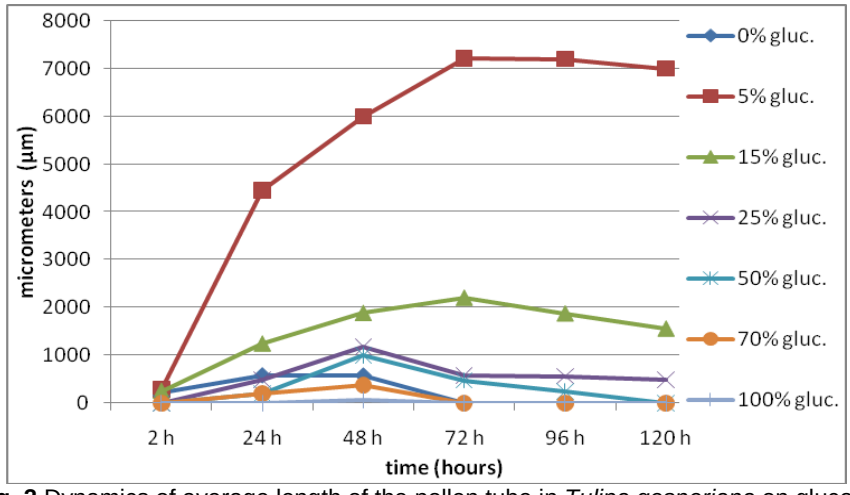


Fig. 2 Dynamics of average length of the pollen tube in *Tulipa gesneriana* on glucose mediums

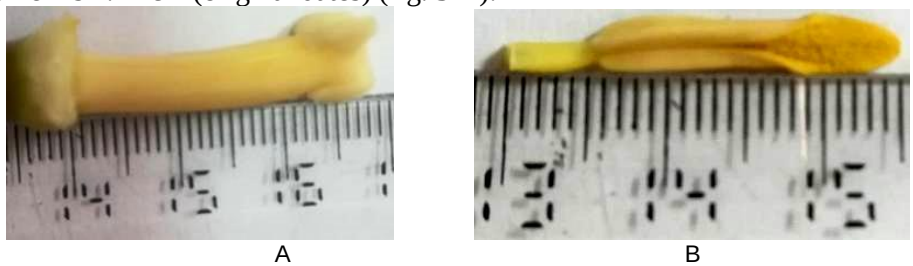
The growth dynamics of tulip pollen tubes demonstrate that hypotonic medium with 5% carbohydrates is optimal for development of male tulip gametophyte necessary for egg fertilization. Of the two carbohydrates used in experiment, sucrose has been shown to be much more effective in providing the longest tulip pollen tubes (fig. 1, fig. 2).

The length of the pollen tube is an essential parameter for the quality of a pollen. This is because the length of pollen tube is closely correlated with length of pistil it penetrates to reach the final destination: the egg.

Tulipa gerneriana ovary is sessile, with an average length of 26.4 mm (original dates) (fig. 3-A).

Given the length of ovary, it is deduced that pollen tubes of *Tulipa gesneriana* must measure at least 26400 μm in length, in order to reach the eggs to achieve double fertilization. Pollen tubes of such lengths were not obtained in present experiment. In this sense, an explanation is required according to which, when pollen is tested *in vitro*, pollen tube grows at a much decreased speed compared to pollen tube increased *in vivo* (Lassig *et al*., 2014).

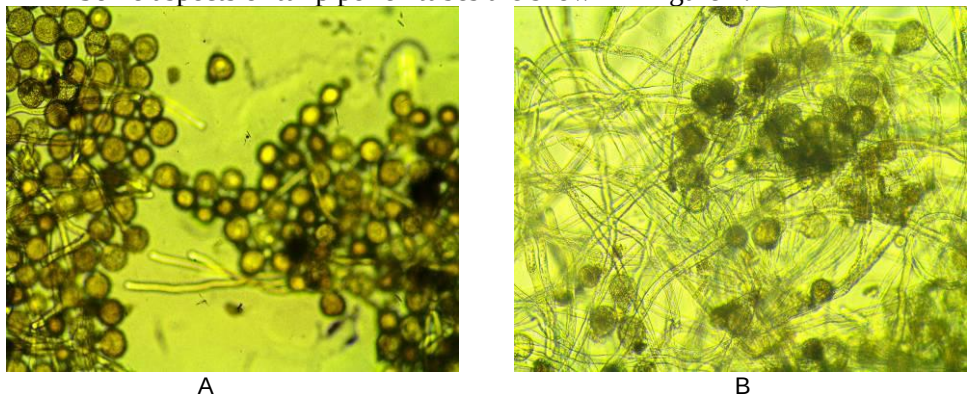
Although tulip is a predominantly vegetative plant, its stamens are large and produce a large amount of pollen. The average value of the length of the tulip stamen is 2.77 cm (original dates) (fig. 3-B).



A B

Fig. 3 The sexual reproductive organs of *Tulipa gesneriana* flower: A – ovary; B - stamina
A division = 1 millimeter (Original)

Some aspects of tulip pollen tubes are shown in Figure 4.



A B

Fig. 4 *Tulipa gesneriana* pollen tubes formed after 2 hours (A) and 24 hours (B) on medium with 5% sucrose from inoculation on nutritive medium (100x) (Original)

CONCLUSIONS

1. The growth rate of the tulip pollen tube depends closely on the type and concentration of carbohydrates in nutrient substrate, as well as on the time from sowing the pollen on nutrient medium.

2. Tulip pollen develops the longest pollen tubes on substrate enriched with sucrose. Glucose is suboptimal for pollen of *Tulipa gesneriana*.

3. The optimal concentration of sucrose that supports the growth of the longest tulip pollen tubes is 5%. This means that a hypotonic 5% sucrose nutrient substrate is very effective in guaranteeing the energy needed for the normal growth of tulip pollen tube.

4. The time required for tulip pollen to grow and for them to cross the ovary to reach the eggs is estimated at 24-48 hours after pollination.

5. The viability of tulip pollen tubes is at least 120 hours from the beginning of their build, a very important aspect in the directed hybridization works.

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