DYNAMICS OF POLLEN TUBE GROWTH IN *HYACINTHUS ORIENTALIS* L.

DINMICA CREŞTERII TUBULUI POLINIC DE *HYACINTHUS ORIENTALIS* L.

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Abstract. *The pollen tube is essential for sexual reproduction of plants, as it ensures the delivery of male gametes cells into ovule. Edification of pollen tube by elongation of vegetative cell from pollen grain, growth rate of pollen tube, its response to orientation signals to ovule micropyle and to other extracellular factors are fascinating aspects of pollen biology, which recommend pollen tube as a model for studying the behavior of plant cells. This study focuses on dynamics of in vitro growth of pollen tube of Hyacinthus orientalis on nutrient mediums with different carbohydrate compositions. 15% sucrose medium ensured growth of the longest pollen tubes exceeding 5000 µm just 24 hours after inoculation. Those pollen tubes increased at least another 96 hours, but at low rates, remaining viable. Pollen tubes grown on glucose mediums were significantly shorter, not exceeding 1080 µm. The length of pollen tube indicates its efficiency in fertilizing ovule.*
Key words: *Hyacinthus orientalis*, nutritive medium, pollen tube, dynamics growth

Rezumat. *Tubul polinic este esențial pentru reproducerea sexuală a plantelor, acesta asigurând livrarea gameților masculini în ovul. Edificarea tubului polinic prin alungirea celulei vegetative din granulul de polen, rata de creştere a tubului polinic, răspunsul acestuia la semnalele de orientare către micropilul ovulului şi la alți factori extracelulari sunt aspecte fascinante ale biologiei polenului, care recomandă tubul polinic ca model pentru studiul comportamentului celulelor vegetale. În prezentul studiu se focalizează dinamica creşterii in vitro a tubului polinic de Hyacinthus orientalis pe medii nutritive diferite prin glucidul din compoziție. Mediul cu 15% zaharoză a asigurat creşterea celor mai lungi tuburi polinice care depăşesc 5000 µm la doar 24 ore de la inoculare. Respectivele tuburi polinice au crescut încă cel puțin 96 ore, însă cu rate scăzute, rămânând viabile. Tuburile polinice crescute pe mediile cu glucoză au fost semnificativ mai scurte, nedepăşind 1080 µm. Lungimea tubului polinic indică eficiența acestuia în fertilizarea ovulului.*
Cuvinte cheie: *Hyacinthus orientalis*, mediu nutritiv, tub polinic, dinamica creşterii

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INTRODUCTION

The pollen tube of flowering plants grows in length at its tip to reach an ovule where it delivers two nonmotile male gametes during double fertilization and initiate seed development. The mechanism of guiding the pollen tube in stylar tissue can be explained by the fact that pollen tubes respond to chemoattractants secreted by unfertilized ovules (Yetisen *et al*., 2011). Pollen is considered an excellent model for the study of gene expression and the growth process of the pollen tube, given that its genome is haploid (Johnson *et al.,* 2019).

Molecular studies using immunofluorescence techniques have shown that transcriptional resumption and pre-mRNA maturation occur during the growth of the pollen tube of *Hyacinthus orientalis*, compared to the anthesis stage of pollen (Zienkiewicz *et al.,* 2006, 2008). In the pollen tube, the sperm nuclei of *Hyacinthus orientalis* have an intense transcriptional activity that ends before fertilization (Zienkiewicz *et al.,* 2011).

The aim of this paper is to highlight the peculiarities of dynamics' growth of the pollen tube in *Hyacinthus orientalis*, as well as the influence of the nutritive medium on it. These investigations also contribute to the completion of the knowledge about sexual reproduction of this genotype known as a valuable ornamental plant, with a short flowering period.

MATERIAL AND METHOD

The biological material is represented by the fresh pollen of *Hyacinthus orientalis* L. For to determine the dynamics of pollen tube growth, we have 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. Were made micromeasurements for determining the dynamics for the 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 8 readings/nutritive medium variant/time interval.

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 Oxion optical microscope.

To determine the length of floral style, it was calculated an average by measuring of 100 style from 100 flowers of *Hyacinthus orientalis*, using the binocular magnifying glass Bel photonics.

RESULTS AND DISCUSSIONS

Two hours after pollen inoculation, the first pollen tubes appeared, only on mediums with 0% - 25% carbohydrates. The longest are the pollen tubes formed on mediums with 15% sucrose (428 μm), and the shortest ones on mediums without carbohydrates (12 μm) (fig. 1, fig. 2).

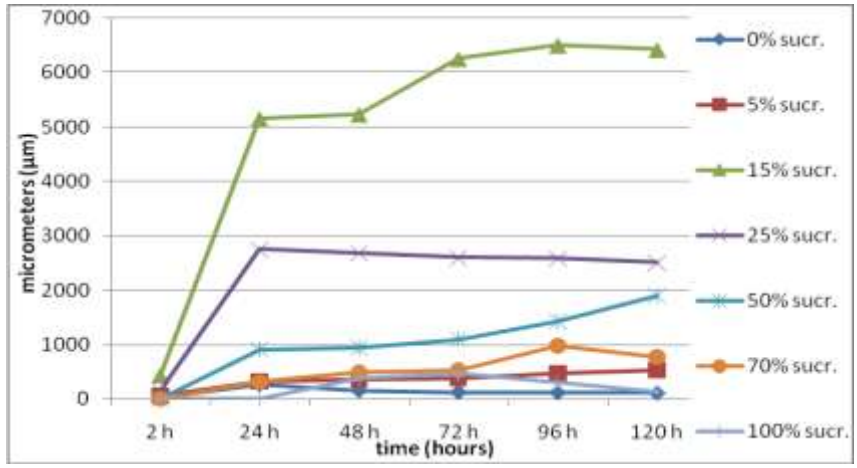

Fig. 1 Dynamics of average length of the pollen tube in *Hyacinthus orientalis* on sucrose mediums

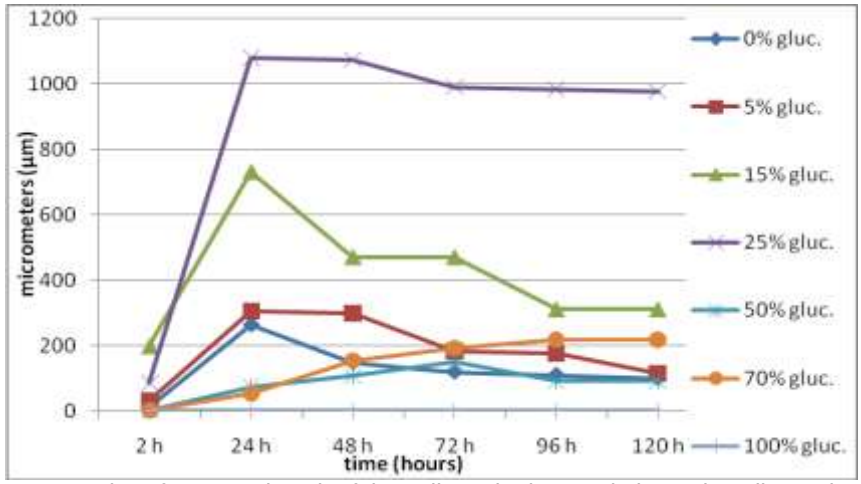

Fig. 2 Dynamics of average length of the pollen tube in *Hyacinthus orientalis* on glucose mediums

At 24 hours after inoculation, the pollen tubes increase significantly in length, especially by on 15% sucrose, when the average length exceeds 5000 μm. There are large differences between the length of pollen tubes grown on sucrose

mediums and those grown on glucose mediums, in favor of those on sucrose mediums (fig. 1, fig. 2).

48 hours after pollen inoculation, pollen tubes grown on sucrose mediums are found to increase in length, but not significantly. The longest tubes are supported by the medium with 15% sucrose. In case of pollen tubes grown on glucose mediums, the situation is different, in sense that they are much shorter than those grown on sucrose mediums. Moreover, some pollen tubes underwent resorption on mediums with 5%, 15% and 25% glucose. Of the glucose mediums, only the 25% glucose variant supports the longest pollen tubes, but they are much shorter than those developed on the 25% sucrose medium (fig. 1, fig. 2).

After 72 hours from inoculation, the evolution of increase in length of the pollen tubes does not differ much from previous time interval. Tubes grown on sucrose mediums grow insignificantly, the longest being those increased on sucrose 15% (6000 μm), and the shortest being those increased on sucrose 5% (375 μm). Glucose mediums continue to be suboptimal for the increase in length of hyacinth pollen tubes. Even 72 hours after inoculation, pollen tubes grown on glucose mediums have no lengths comparable to those on sucrose. On the contrary, there are regressions of their length due to degeneration by breaking them at the tip. There is a slight increase in the tubules on the media with 50% and 70% glucose. Medium with 100% glucose does not allow the growth of any pollen tubes (fig. 1, fig. 2).

96 hours after inoculation, pollen tubes of *Hyacinthus orientalis* are still viable in both categories of nutritive mediums.The tubes grown on sucrose mediums are still elongating, but at insignificant speeds compared to the previous interval. The pollen tubes grown on glucose remain significantly shorter than those grown on sucrose (fig. 1, fig. 2).

120 hours after inoculation, hyacinth pollen tubes, especially those grown on sucrose mediums, are still viable, whole. The nutritive medium with 100% glucose did not allow the growth of pollen tubes even after five days from pollen inoculation (fig. 1, fig. 2).

The dynamic analysis of the growth of pollen tube of *H. orientalis* during the 120 hours of observation highlights the following aspects (fig. 1, fig. 2).

In the first 24 hours after pollen inoculation on nutrient mediums, was the most significant increase in pollen tubes, on most mediums variants, but with different rates.

Mediums with 0%, 5% and 100% carbohydrates (sucrose and glucose) were inhibitory mediums for the growth of hyacinth pollen tubes.

Mediums enriched with 50% and 70% carbohydrates proved to be suboptimal mediums because they do not allow the normal elongation of pollen tubes of *H. orientalis*.

The 15% sucrose medium has been shown to be optimal for expressing the maximum growth potential of *H. orientalis* pollen tubes. On this medium, the pollen tubes reached over 6000 μm in length.

Of the nutrient mediums with glucose, only that with 25% concentration supported the longest pollen tubes (about 1000 μm in length).

Analysis of the growth dynamics of hyacinth pollen tubes shows that hypotonic (0-5% carbohydrates) and hypertonic (50-100% carbohydrates) mediums are suboptimal for egg fertilization.

The optimal mediums for growing hyacinth pollen tubes are those with 15% and 25% carbohydrates, but the type of carbohydrate matters a lot. So 15% sucrose is much better for hyacinth pollen than 15% and 25% glucose.

Pollen quality is ensured by two parameters: germination capacity and pollen tube length. The pollen tube must be long enough to reach the ovule, to which it will deliver the two sperm cells. In this sense, the normal length of pollen tube is in direct correlation with length of the flower style and ovary which it penetrates to reach the ovule (Harder *et al.,* 2016). In *H. orientalis*, the average length of style is 1.62 mm, and that of ovary 3.54 mm (original dates) (fig. 3). Considering the length of style and ovary of pistil, it follows that the pollen tubes in *H. orientalis*, in order to reach ovules, must be at least 5000 μm long.

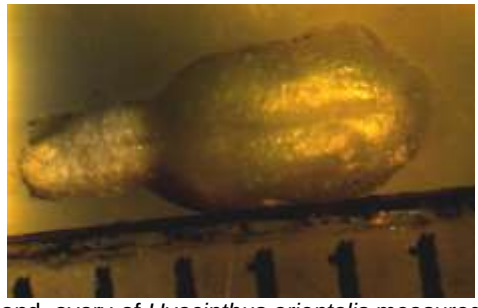

Fig. 3 The floral style and ovary of *Hyacinthus orientalis* measured by binocular loupe. A division = 1 millimeter (Original)

In figure 4 are presented aspects of pollen tubes of *H. orientalis* grown on mediums with 15% sucrose.

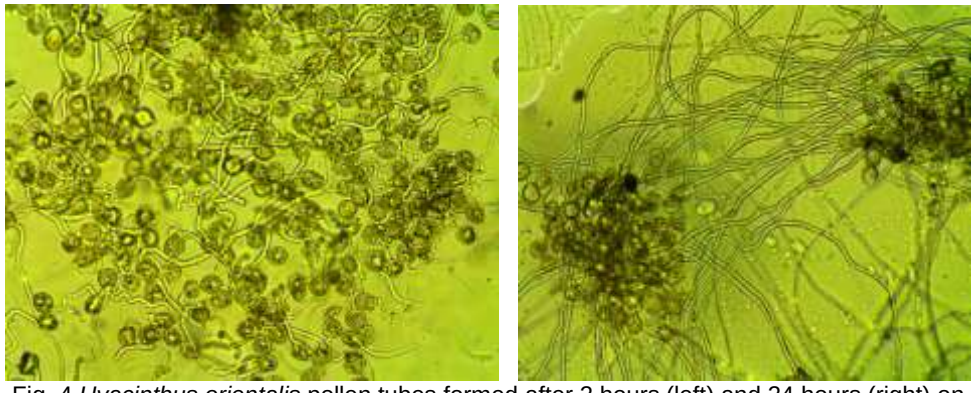

Fig. 4 *Hyacinthus orientalis* pollen tubes formed after 2 hours (left) and 24 hours (right) on medium with 15% sucrose from inoculation on nutritive medium (100x) (Original)

CONCLUSIONS

1. The present experiment showed that the length of pollen tubes varies depending on three factors: the type of carbohydrate in nutritive medium, the concentration of carbohydrate in nutritive medium and the time elapsed since the inoculation of the pollen on nutritive medium.

2. Sucrose has been shown to be preferred to glucose for the optimal development of the male gametophyte in *Hiacynthus orientalis*. This fact suggests that the energy required for pollen tube growth of hyacinth can be provided by successfully supplied by sucrose which is much more efficient than glucose.

3. The optimal concentration of sucrose required to express the maximum length of the pollen tube of *Hyacinthus orientalis* is 15%.

4. The time required for *Hyacinthus orientalis* pollen tube to reach the optimal length that allows it to reach the ovule is 24 hours.

5. *Hyacinthus orientalis* pollen tubes are viable for at least 120 hours from the time of their construction.

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