

POLLEN TUBE GROWTH RATE AND MORPHOLOGICAL ANOMALIES OF POLLEN TUBES AT *SCILLA BIFOLIA* L.

RATA DE CREȘTERE A TUBULUI POLINIC ȘI ANOMALIILE MORFOLOGICE ALE TUBURILOR POLINICE DE *SCILLA BIFOLIA* L.

PĂDUREANU Silvia¹

e-mail: silvyp27@yahoo.com

Abstract. *This study of male gametophyte of Scilla bifolia L. ssp. bifolia highlights pollen tube growth and growth rate which are central role in the fertilization process for plants. Reference is made to the cytological aspects of pollen tubes in relation with sucrose concentration in culture mediums. We used variants of nutrient media, with sucrose concentration of 0% to 100%. We conclude that is a strong positive correlation between the increase in length of pollen tubes and germination potential. The longest pollen tubes were formed on mediums with 15% - 25% sucrose. The data obtained prove that the increase in pollen tube length is genetically determined being correlated with the length of floral style. The growth rate of pollen tubes of Scilla bifolia is accelerated in the first 24 hours after inoculation pollen. We highlight the peculiarities of building pollen tube, longevity and abnormalities in male gametophyte development of Scilla bifolia.*

Key words: *Scilla bifolia*, nutritive medium, pollen tube growth, abnormalities

Rezumat. *Prezentul studiu al gametofitului mascul de Scilla bifolia L. evidențiază creșterea tubului polinic și rata lui de creștere, aspecte cu rol central în fertilizarea plantelor. Se face referire asupra aspectelor citologice ale tuburilor polinice în corelație cu concentrația în zaharoză din mediile de cultură. S-au folosit variante de medii nutritive cu concentrații în zaharoză de 0% până la 100%. Concluzionăm că există o strânsă corelație pozitivă între creșterea în lungime a tuburilor polinice și potențialul germinativ. Cele mai lungi tuburi polinice la acest taxon s-au format pe mediile cu 15%, 20% și 25% zaharoză. Datele obținute probează că procesul de creștere în lungime a tuburilor polinice este determinat genetic, fiind corelat cu lungimea stilului floral. Ritmul de creștere a tuburilor polinice de Scilla bifolia este accelerat în primele 24 ore de la inocularea polenului. Se evidențiază particularitățile edificării tubului polinic, longevitatea și anomaliile în dezvoltarea gametofitului mascul la Scilla bifolia.*

Cuvinte cheie: *Scilla bifolia*, mediu nutritiv, creșterea tubului polinic, anomalii

¹University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

INTRODUCTION

For sexual reproduction of flowering plants, the pollen is very important because delivers the sperm to the egg. As soon as the pollen on the stigma landed, if the pollen–stigma interaction is compatible, the pollen grain hydrates and germinates. The pollen tube growth is a complex process involving enzymatic systems, molecular motors - dyneins and kinesins, synthesis of cellulose for its wall. Such researches its have made in *Nicotiana tabacum*, *Nicotiana glauca*, *Arabidopsis*, *Lilium longiflorum* (Krichevsky *et al.*, 2007). Rapid elongation of the pollen tube, over a distance of a few hundred micrometers to several centimeters, is an essential process for reproductive success of flowering plants. In *Arabidopsis* it has been found that the efficient elongation of the pollen tube requires a K⁺ channels activated of specific genes (Mouline *et al.*, 2002). Recently researches in three water lilies (*Nymphaea odorata*, *Nuphar advena*, *Brasenia schreberi*) evaluated the pollen tube growth rate through changes in the volume of wall material used for growth. Thus, it proved that there is a tradeoff between increasing efficiency and other features of the pollen tube (Williams *et al.*, 2016).

In the present study, we investigate the growth rate, morphological peculiarities and anomalies of pollen tube of *Scilla bifolia* L., like the knowledge base for improvement breeding, knowing that this genotype is wortf of ornamental plant with blooming period of approx. 23 days from the end of March, offering the first nectar for bees (Žuraw, 2011).

MATERIAL AND METHOD

The biological material is represented by pollen of *Scilla bifolia* L. ssp. *bifolia*. Pollen was sampled at the anthesis phase. The pollen germination process was determined with hanging drop (Erdman, 1952). So, pollen grains have been inoculated on agar nutritive mediums (1%), at which sucrose was added at different concentrations: 0%, 5%, 10%, 15%, 20%, 25%, 40%, 50%, 70%, 100%. Ten experimental variants resulted. For each experimental variant, we have used 10 “wet rooms”. The quantity of pollen inoculated on these mediums was the same in all the cases. Were made micromesurements for determining the dynamics for the extension of pollen tubes. In this respect, the readings at microscope were done at 1.5, 24, 48, 72, 120, and 144 hours since the inoculation of the pollen grains on nutritive medium. Micromesurements 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 Hund Wetzlar optical microscope. To determine the length of floral style, it was calculated an average by measuring of 100 style from 100 flowers of *Scilla bifolia*, using the binocular magnifying glass Bel photonics.

RESULTS AND DISCUSSIONS

Results related to the average length of pollen tubes of *Scilla bifolia* are registered in table 1.

The average length of the pollen tube (μm) in *Scilla bifolia* ssp. *bifolia*

| Time of inoculation | % sucrose in medium | | | | | | | | | |
|---------------------|---------------------|------|------|------|------|------|-----|-----|-----|------|
| | 0% | 5% | 10% | 15% | 20% | 25% | 40% | 50% | 70% | 100% |
| after 1.5 hours | 238 | 242 | 285 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| after 24 hours | 490 | 497 | 590 | 3298 | 3447 | 3353 | 280 | 0 | 0 | 0 |
| after 48 hours | 852 | 972 | 1101 | 3878 | 3809 | 3470 | 625 | 325 | 234 | 93 |
| after 72 hours | 678 | 1003 | 1189 | 3890 | 4180 | 3491 | 439 | 502 | 311 | 343 |
| after 120 hours | 0 | 1112 | 1207 | 3874 | 4241 | 3404 | 428 | 495 | 309 | 217 |
| after 144 hours | 0 | 1174 | 1211 | 3790 | 4202 | 3291 | 385 | 488 | 210 | 203 |

It finds that the length (expressed in micrometers) of pollen tubes varies depending on two factors: carbohydrate composition of the nutritive medium and the time elapsed from the time of inoculation of pollen on medium. After the first 1.5 hours after inoculation of pollen, appeared the first pollen tubes only on the poorest mediums in sucrose, namely those with 0%, 5% and 10% sucrose. In this case, the pollen tubes not exceeding 300 μm in length. 24 hours after inoculation, there is an explosive growth of pollen tubes on mediums enriched with 15%, 20% and 25% sucrose. On these three mediums, the length of pollen tube exceeds 3000 μm . Also, in this time appeared pollen tubes on medium with 40% sucrose, but very short, only 280 μm . On mediums low in sucrose, the pollen tubes elongate very little. After 48 hours from inoculation, on all variants of nutritive mediums are formed of pollen tubes. The longest tubes are those grown on mediums with 15%, 20% and 25% sucrose. Compared to the previous period, at this point the pollen tubes were increases in length of in all cases. We emphasize that only now appeared the pollen tubes on mediums highly concentrated in sucrose. After 72 hours of inoculation occur varied changes depending on the composition of culture medium. Thus, the absence of sugar in medium, does not allow the survival of pollen tubes, they breaking at tip and resorbing. Therefore, the average length of pollen tubes decreases. The same phenomenon occurs in the variant with 40% sucrose in the medium. On other mediums, pollen tubes grow slightly in length. After 120 hours, on medium without sucrose all pollen tubes are resorbed. On the other mediums, the transformations are insignificant compared to the previous period. After 144 hours prevail resorption processes of pollen tubes to majority variants, therefore values are in decline. Only on mediums with 5% and 10% sucrose, pollen tubes increase, but insignificant.

The dynamic analysis of the growth rate of length tubes pollen of *Scilla bifolia* (fig. 1) shows that in first 24 hours after inoculation pollen on culture mediums, there is a significant jump of elongation of tubes, but only on three

mediums, namely those with 15%, 20% and 25% sucrose. On these nutritive mediums, pollen tubes exceeded 3000 μm . In following periods, rate of growth of pollen tubes grows on the three mediums in general, but not significantly (fig.1).

It result that hypotonic (0-10% sucrose) and hypertonic (with 40-100% sucrose) mediums are not effective in case *Scilla bifolia* for fertilizing ovules. The presented results proved that the pollen's viability of *Scilla bifolia* is accentuated.

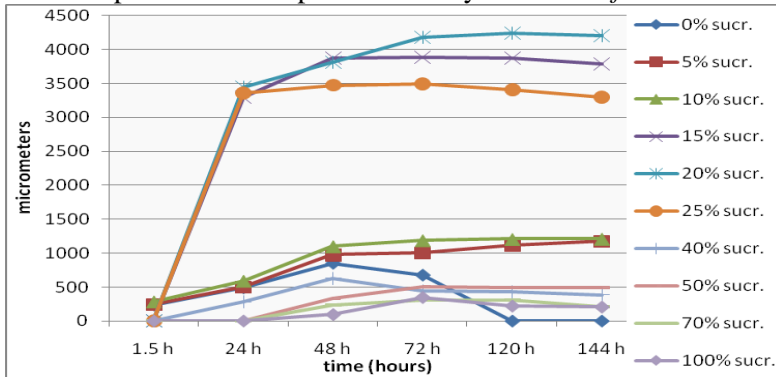


Fig. 1 Dynamics of average length of the pollen tube in *Scilla bifolia*

Pollen tube length is always correlated with the length flower style. To *Scilla bifolia* flower style is 2800 μm (fig. 2). This value is an average of measurements from 100 styles flowers. It follows that only pollen tubes at least 2800 μm length can realize fertilizing the ovules. Bat a low percentage of pollen tube development, despite the high fertility of pollen grain of *Arbutus andrachne* and *Osmanthus decorus* determine the embryo degeneration (Melia *et al.*, 2012).

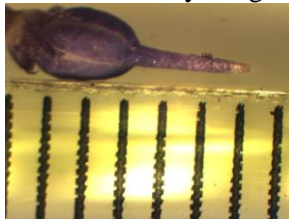


Fig. 2 The floral style of *Scilla bifolia* measured by binocular loupe. A division = 1 millimeter

In figures 3-5 are presented aspects of pollen tubes of *Scilla bifolia* formed on mediums with different concentrations of sucrose.

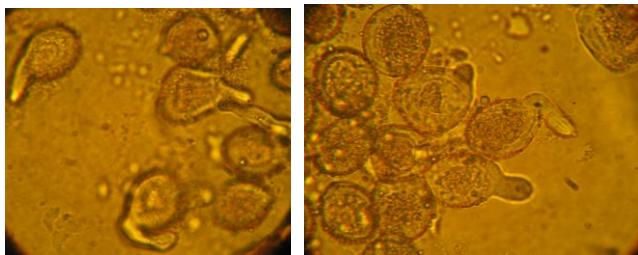


Fig. 3 Beginning of edification of pollen tube in *Scilla bifolia* (400x) (Original)



Fig. 4 *Scilla bifolia* pollen tubes formed after 1.5 hours on medium with 0% sucrose (left), on medium with 5% sucrose (center) and on medium with 10% sucrose (right). Arrow indicates a broken tube at top (400x) (Original)

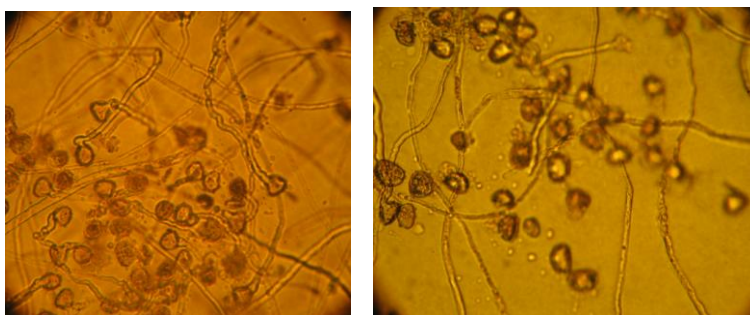


Fig. 5 *Scilla bifolia* pollen tubes formed after 24 hours on medium with 15% sucrose (left) and on medium with 20% sucrose (right) (100x) (Original)

Is concluded that on nutritive mediums with longest pollen tubes, germination percentage is high.

The pollen germination process of *Scilla bifolia* was marked by the occurrence of atypical forms of pollinic tubes, known in literature as abnormalities. These consisted in: dilatations at top and basis pollen tube (fig. 6); tubes which are branched at the basis or at middle, or at top (fig. 7, fig. 8).



Fig. 6 *Scilla bifolia* pollen tubes with dilatation at top (left, center) and at basis (right). Arrows indicate the dilatations (400X) (Original)

Dattilo *et al.* (2005) show that the *Actinidia deliciosa* pollen tubes suffer morphological anomalies under the influence of 50 Hz magnetic field, which induces helicoidale pollen tubes, because Cl and Ca ions flux change in cytoplasm of the pollen tubes.

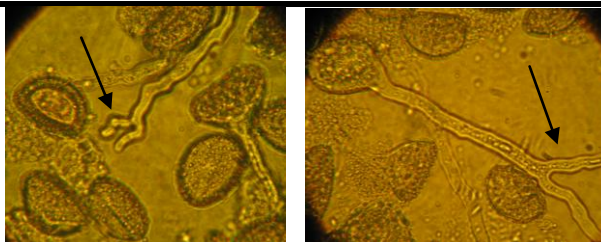


Fig. 7 *Scilla bifolia* pollen tubes branched at top. Arrows indicate the ramifications (400X) (Original)

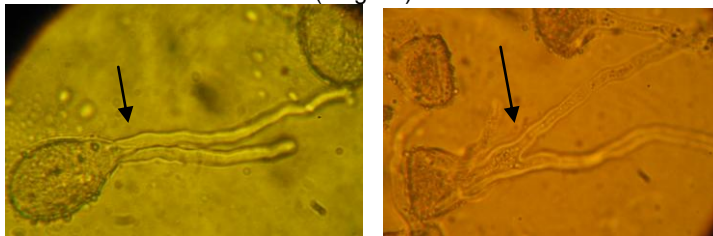


Fig. 8 *Scilla bifolia* pollen tubes branched at basis (left) and at middle (right). Arrows indicate the ramifications (400X) (Original)

CONCLUSIONS

1. At *Scilla bifolia*, able pollen tubes for fertilize ovules are those formed on mediums with 15-25% carbohydrates concentration, because only these concentrations ensure their growth in length at maximum, being able to cross the stylar tissue to reach ovules.

2. Pollen tube growth rate of *Scilla bifolia* is maximum in the first 24 hours after inoculation, this aspect being available for most plants.

3. Some of *Scilla bifolia* pollen tubes have morphological abnormalities characteristic of this genotype, whose cause remains unknown for now.

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