

## THE PALYNOLOGICAL CHARACTERIZATION AND THE POLLEN GERMINATION PROCESS OF LILY HYBRID *STAR GAZER*

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### Abstract:

This paper presents the morphological characteristics of pollen of lily, hybrid Star Gazer: shape of pollen grains, exine ornamentation, size of pollen grain and number of germinative pores/pollen grain. Also on presents the characteristics of pollen germination process of the lily, hybrid Star Gazer. In this regard were carried pollen germination tests "in vitro" in wet rooms "van Tieghem." The nutrient medium that were used for inoculation of the pollen grains had different concentrations of sucrose, resulting several experimental variants. The pollen germination capacity was determined from time to time, thereby establishing the dynamics of this process for four days. Surprising was the fact that the highest values of germination potential were realized on medium without sugar. On the nutrient mediums with a moderate concentration in sucrose, the germination capacity of the hybrid lily was inhibited. During the first 24 hours after inoculation of the medium pollen occurs a significantly improved the pollen germination rate. In parallel with the determination of pollen germination capacity was analyzed and increasing the length of pollen tubes. This dynamic was also analyzed for four days. The longest pollen tubes were formed on the medium without sugar. As in the case of the germinating potential, after 24 hours from the inoculation of the pollen on medium has achieved a significant elongation of pollen tubes. Also, there is a direct and close correlation between the pollen tube length and the flower style length of the of which that they have to traverse in order to reach the ovul. The length of *Lilium Star Gazer* pollen tubes is positively correlated also with germination rate.

**Key words:** *Lilium Star Gazer*, pollen grain, nutritive medium, pollen germination, pollen tube

*Lilium Star Gazer* is classified as Oriental lilies, is adapted to colder climates (Sun M. et al., 2011). This taxon is diploid with  $2n = 2x = 24$  chromosomes (Marasek A., Orlikowska T., 2003).

The pollen of some lily genotypes was investigated with regard to storage and viability and it was concluded that the storage in the dark chamber where the temperature was maintained less than  $-20^{\circ}\text{C}$  for one year ensure a sufficiently high percentage for germination of pollen (Rhee H.K. et al., 2003).

The *Lilium longiflorum* pollen tube constituted an excellent experimental model for the improvements of the ultrastructural detail of plant cell use of rapid freeze fixation and freeze substitution (RF-FS) (Lancelle, S. A., Hepler, P. K., 1992). They are easily cultivated in vitro and have a very dynamic cytoskeleton that polymerizes at very high rates, providing the pollen tube with interesting mechanical properties. The pollen tube has an unusual kind of growth, it extends exclusively at its apex. Extending the cell wall only at the tip minimizes friction between the tube and the invaded tissue. This tip growth is performed in a pulsating manner rather than in a steady fashion. Pollen tube growth stops for

several minutes before resuming. Subsequent growth has a lower mean rate, but continues to oscillate, albeit with a longer period.

Oscillatory tip growth in pollen tubes of *Lilium formosanum* was highlighted by Rounds C.M. et al. (2010) who concluded that pollen tube growth oscillations are the result of a mechanism that focuses mitochondria in the apical region of the tube.

Also energy lily pollen tube allowed to explain the mechanisms of rapid growth based on high rates of respiration (Dickinson D., 1965 Rounds C.M. et al., 2011). Pollen tubes are easily grown in vitro and have become an excellent model for investigating the contributions of respiration to plant cellular growth and morphogenesis at the molecular, biochemical and physiological levels.

In this paper we intend to investigate the morphological characteristics of pollen and pollen germination of *Lilium Star Gazer* considering that about this hybrid taxon has not been such research.

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## MATERIAL AND METHOD

The biological material is represented by the fresh pollens of *Lily* hybrid *Star Gazer* were employed as experimental material to study the palynological characterization and the germination process. In order to define the pollen morphology, we determined shape of pollen grains, exine ornamentation, size of pollen grain and number of germinative pores/pollen grain. For determining the shape of pollen grains and of exine ornamentations, we have used the Hund Wetzlar microscope, at which we took microphotographs.

For determining the size of pollen grains we did micromerements at 1000 grains. We measured the longitudinal and the equatorial diameter. 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 consisted in introducing the pollens in a mixture of concentrated sulphuric acid, acetic acid, methylene blue. For study the the germination process, we have used the so-called van Tieghem "wet rooms". The nutritive mediums necessary for the germination of pollens consisted in distilled water, agar 1% and sucrose at different concentrations: 0%, 5%, 10%, 15%, 25%, 35%, 45%, 70% and 100%. Thus, 9 experimental variants resulted. For each experimental variant, we have used 10 "wet rooms". The amount of inoculated pollen per each medium was the same in all cases. Readings at the Hund Wetzlar optic microscope were done at 1, 24, 48, 72 and 96 hours since the pollen inoculation on 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.

In parallel were made micromerements for determining the dynamics for the extension of pollen tubes. For pointing out the characteristics of pollen tube from this taxon, photographs were taken at Hund Wetzlar optical microscope.

## RESULTS AND DISCUSSIONS

### The palynological characterization of *Lilium Star Gazer*

Pollen of *Lilium Star Gazer* is elliptical, yellow-orange, with the reticulate exine (fig. 1, 2). Each grain has an aperture represented by a trench that connecting the two poles (fig. 1, 2).

The polar axis has an average of 147.936  $\mu\text{m}$  and the equatorial axis is 64.2715  $\mu\text{m}$ . The ratio between the two diameters was around 2.3. The coefficient of variation (s%) show little variability for the polar axis and middle variability for equatorial axis (tab. 1).

Pollen of *Lilium Star Gazer* has in all cases analyzed a single germinative pore/grain (fig. 3). All liliates have a single germinative pore/pollen grain.

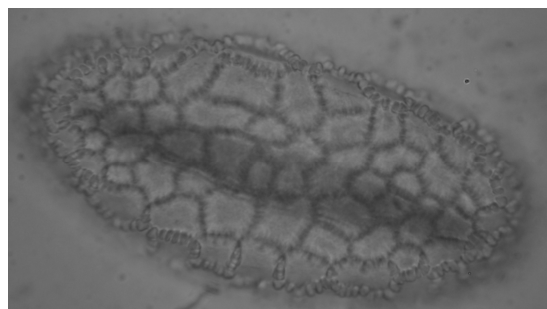


Figure 1 Pollen grain of *Lilium Star Gazer* (1000X) (Original)



Figure 2 Pollen grain of *Lilium Star Gazer* (400X) (Original)

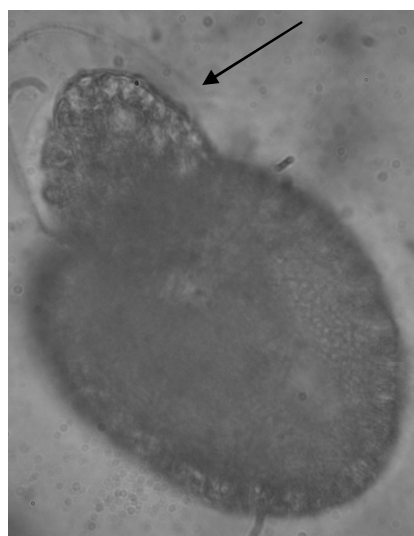


Figure 3 Germinative pore (indicated by the arrow) at pollen grain of *Lilium Star Gazer* (1000X) (Original)

After one hour since inoculation on artificial mediums, the pollen of *Lilium Star Gazer* is germinating only on medium without the addition of sucrose, in percentage 47%. On other experimental mediums, the pollen grains not are germinating (tab. 2).

After 24 hours after inoculation, pollen is germinating in a proportion of 93% on medium without sucrose, and in proportions lower on mediums enriched with sucrose in a concentration of 5%, 10%, 15%, 25% and 35% (tab. 2).

Table 1

Variability of pollen grain size in *Lilium Star Gazer*

Type of diameter	Mean value (μm)	Minimum value (μm)	Maximum value (μm)	Variation height (μm)	S (μm)	S%	$\bar{Sx}$ (μm)	Rate high diameter/ equatorial diameter (μm)
high diameter	147.9360	110.40	175.95	65.55	14.754	9.973	1.4754	2.3017
equatorial diameter	64.2715	48.30	93.15	44.85	8.360	13.01	0.836	

The pollen germination process of *Lilium Star Gazer*

Within 48 hours after inoculation of pollen is an increase in the percentage of germinated pollen on all variants of artificial mediums. On the medium without of sucrose is the highest rate of pollen germination (tab. 2). After 48 hours, the lily pollen is germinating on mediums with 45%, 70% and 100% sucrose, but in small proportions.

After three days (72 hours) since inoculation, the percentage values increased insignificant on all experimental variants of the medium. The highest rate of germination capacity is achieved on the medium without of sucrose. It keeps the same negative correlation between the percentage of germinated pollen grains and the

percentage of sucrose in artificial mediums (tab. 2).

After four days (96 hours) after inoculation on nutrient mediums, pollen is still viable, as evidenced by increases in the percentage of pollen grains on most experimental mediums. But these increases are not significant. On the medium with 0% sucrose maintains the same high level of germinating potential. It is still evident that the sucrose in the culture mediums inhibit on the germination process, to varying degrees, depending on its concentration (tab. 2).

Similar results were obtained by studying pollen germination of *Hippeastrum vittatum*, which belongs to the family *Amaryllidaceae* (Pădureanu S., Cireașă E., 1995).

Table 2

The pollen germination (%) of *Lilium Star Gazer* in mediums with different concentrations of sucrose

the time of inoculation	% sucrose in medium								
	0%	5%	10%	15%	25%	35%	45%	70%	100%
after 1 hour	47	0	0	0	0	0	0	0	0
after 24 hours	93	44	36	35	33	30	0	0	0
after 48 hours	94	65	61	57	54	49	43	39	32
after 72 hours	95	77	76	72	68	63	47	46	41
after 96 hours	95	82	80	79	79	65	61	55	43

The germination of *Lilium* pollen on medium without sugar was confirmed by Qiu J.Y. et al. (2012), Geng X. et al. (2013). The dependence of germination and pollen tube growth on sugar sources varies with plant species. Maltose and sucrose were suboptimal sugar source for *Lilium oriental* „Sorbonne” and *Lilium davidii* (Qiu J.Y. et al., 2012).

Starch was detected in *Lilium* pollen, but not in *Gladiolus* pollen. Contents of sucrose, glucose and fructose in *Lilium davidii* pollen were significantly higher than that in *Gladiolus gandavensis* pollen. *Lilium* pollen could germinate in culture medium only containing PEG. When the pollens were cultured in PEG4000+H<sub>3</sub>BO<sub>3</sub>, the amylase activity of *Lilium* pollen was higher than

that of *Gladiolus* pollen. With the rapid growth of pollen tube, glucose content decreased. The pollen germination rate in PEG culture medium and the independence of *Lilium* pollen germination on sugar sources may be related to of carbohydrate type, content and enzyme activity in nature and germinating pollen (Geng X. et al., 2013).

The dynamic analysis of *Lilium Star Gazer* pollen germination show that after 24 hours of inoculation on medium without sugar, there is a significant jump germination level, then it remains almost constant for next 72 hours (fig. 4). In the other experimental mediums, the pollen germination rate marks an ascending trend during the 96 hours of observation, but without touching

the maximum germination percentage facilitated by the medium without sugar (fig. 4).

The increase in the length of pollen tubes of *Lilium Star Gazer*

The growth process of pollen tubes was pursued in parallel with the germinating potential.

After one hour since the inoculation medium pollen were first small hills and pollen tubes on the medium with 0% sugar concentration. The average length of these pollen tubes was 159 μm. On the mediums enriched with sucrose, the pollen tubes not yet appear after this time (tab. 3).

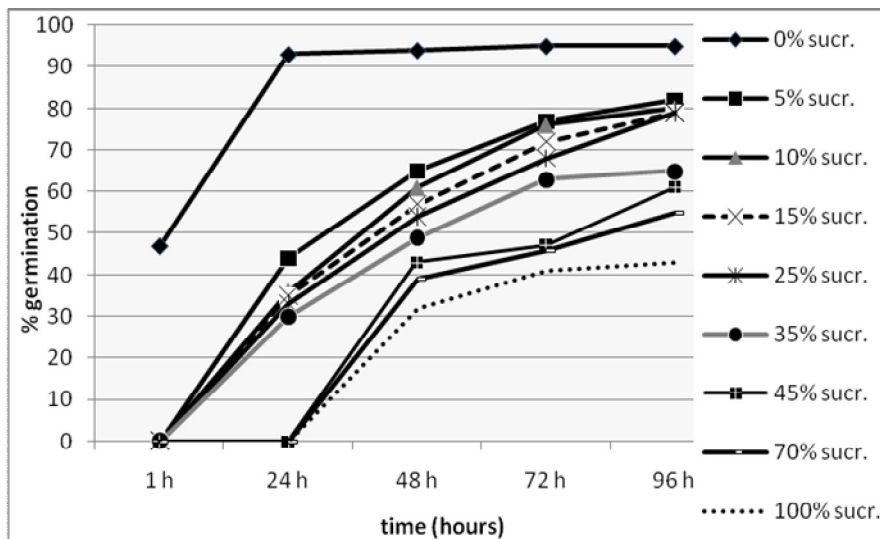


Figure 4 Germination dynamics of *Lilium Star Gazer* pollen

After 24 hours since the inoculation, there was a significant increase in the length of pollen tubes on medium deficiency in sucrose, their mean value reaching 37870 μm. On the experimental mediums with 5-35% sucrose appeared pollen tubes, but much shorter. On mediums with 45-100% sucrose, not yet formed pollen tubes (tab. 3).

After 48 hours since the inoculation, the pollen tubes were elongated insignificant on all variants of mediums. The pollen tubes occurred on mediums enriched with 45-100% sucrose To maintain the same negative correlation between the length of pollen tubes and the concentration of sucrose in the medium (tab. 3). After 72 hours since the inoculation, the pollen tubes were elongated on all mediums variants, the longest being on medium deficiency in sugar, and the shortest, on medium with the highest concentration of sugar. Note that on the medium

sugar free elongation rate is emphasized by this time (tab. 3). After 96 hours since the inoculation, pollen tubes are still viable and were elongated with reduced speeds on the mediums enriched with 15-100% sugar, and faster speeds on mediums with 0-10% sugar (tab. 3).

The dynamic analysis of growth in length of pollen tubes of *Lilium Star Gazer* clearly demonstrates that this process is influenced by sucrose concentration in nutrient media (fig. 5). It shows that the medium lacking sugar is best suitable for pollen tube elongation in a long upward trend, compared to other types of mediums enriched with sucrose. Therefore, on the medium lacking sugar, the pollen tube reaches a maximum of almost 90000 μm. It was noted that on the medium without sucrose, pollen tubes tend to elongate over the limit of the suspended droplet, by filiform extensions (fig. 11).

Table 3

The average length of the pollen tube (μm) at *Lilium Star Gazer* in mediums with different concentrations of sucrose

the time of inoculation	% sucrose in medium								
	0%	5%	10%	15%	25%	35%	45%	70%	100%
after 1 hour	159	0	0	0	0	0	0	0	0
after 24 hours	37870	19220	14140	11848	9370	7217	0	0	0
after 48 hours	47705	30250	28167	24257	10182	8584	7563	4491	2293
after 72 hours	65752	34103	31512	29340	13990	9173	6301	5952	2961
after 96 hours	89402	61951	57508	40710	22580	10502	9105	6777	3079

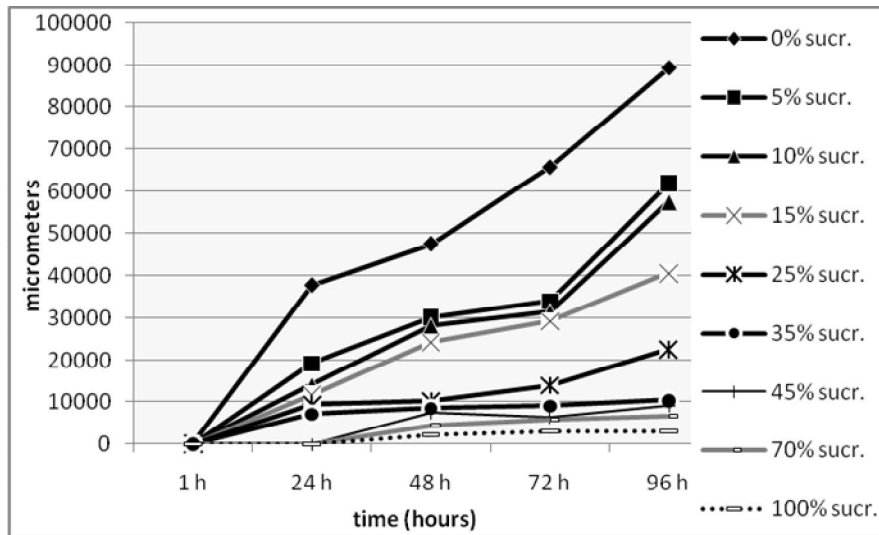


Figure 5 Dynamics of average length of the pollen tube at *Lilium Star Gazer*

Regarding the pollen tube growth in length, should be noted that there is always a direct correlation between flower style length and pollen tube length. In this case, the style of *Lilium Star Gazer* has an average length (obtained by measuring of 100 styles) of 89390  $\mu\text{m}$ . Therefore, only the pollen tube of at least 89390  $\mu\text{m}$  length can reach to the embryo sac for to make the double fertilization.

Also noted the viability of pollen tubes *Lilium Star Gazer* that even after 96 hours after inoculation of pollen, they continue to grow in length, especially on medium without sucrose.

The different aspects of *Lilium Star Gazer* pollinic tubes, formed on different nutritive mediums are presented in figures 6-11.

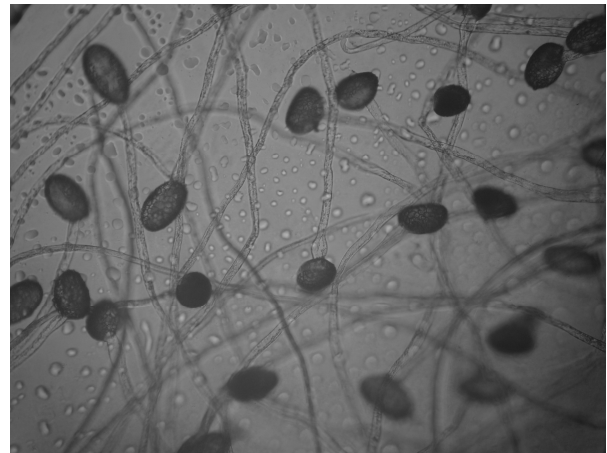


Figure 7 Pollen germination on 0 % sucrose medium, 24 hour after inoculation in *Lilium Star Gazer* (100X) (Original)

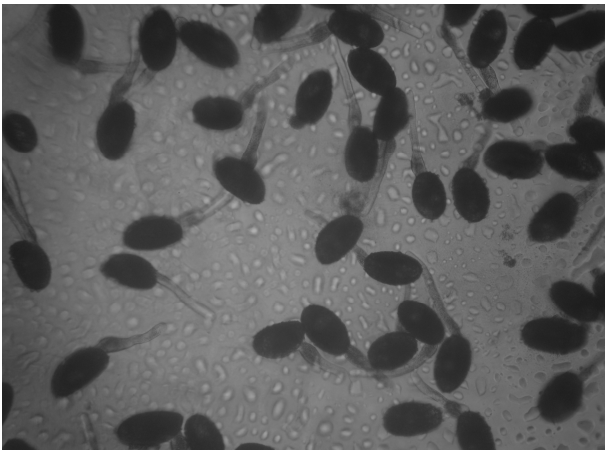


Figure 6 Pollen germination on 0 % sucrose medium, 1 hour after inoculation in *Lilium Star Gazer* (100X) (Original)

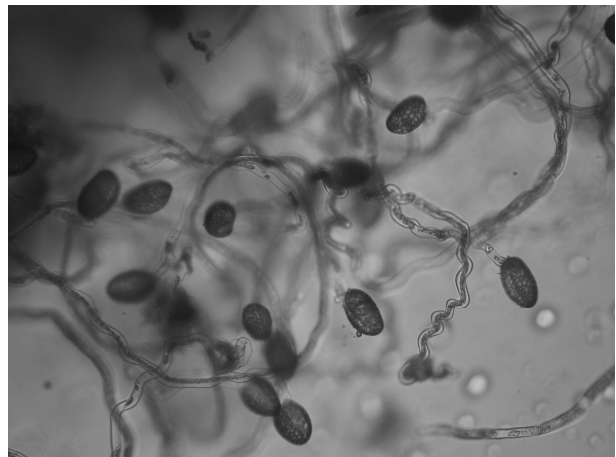


Figure 8 Pollen germination on 25 % sucrose medium, 24 hour after inoculation in *Lilium Star Gazer* (100X) (Original)



Figure 9 Pollen germination on 0 % sucrose medium, 48 hour after inoculation in *Lilium Star Gazer* (100X) (Original)

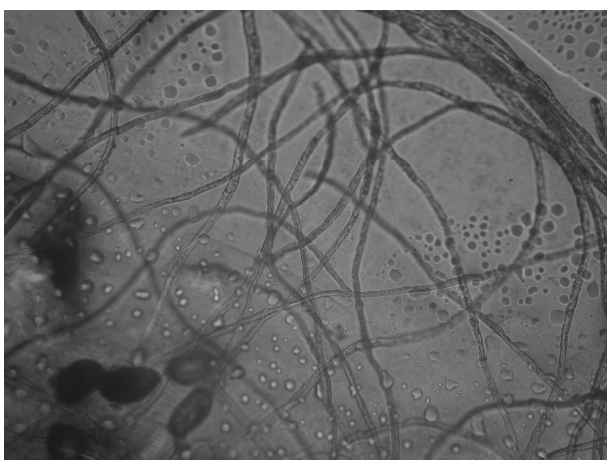


Figure 10 Pollen germination on 0 % sucrose medium, 72 hour after inoculation in *Lilium Star Gazer* (100X) (Original)



Figure 11 Pollen germination on 0 % sucrose medium, 96 hour after inoculation in *Lilium Star Gazer*; the pollen tubes exceed the medium droplet periphery suspended due to their length (100X) (Original)

## CONCLUSIONS

Pollen of *Lilium Star Gazer* has relatively uniform size, which indicates good fertility.

The very high pollen germination rate, over 90% of the pollen of *Lilium Star Gazer* is a proof that the meiosis by the hybrid genotype is normal.

The high germinating potential of *Lilium Star Gazer* pollen expressed only on medium without sucrose. This confirms the literature that explains this phenomenon by high carbohydrate and enzyme content of lily pollen.

Between the increase in the length of pollen tubes of *Lilium Star Gazer* and sugar concentration in medium for germination there is an inverse correlation. Thus, the longest the pollen tubes were formed on medium without sugar. Only these can ensure the fertilization of the ovules. But, between the length of pollen tubes and pollen germination rate is positively correlation.

Pollen tube growth rate is higher in the first 24 hours after inoculation of pollen on nutrient media.

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