CYTOLOGY OF THE GERMINATING PROCESS OF *TRIFOLIUM MONTANUM* L. AND *TRIFOLIUM PANNONICUM* JACQ. POLLEN

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The paper presents the characteristics of the germination process in Trifolium montanum L. and T. pannonicum Jacq. pollen. We made estimates on the length of pollinic tubes in the dynamics of germination (after 3, 24, 48 and 72 hours since the inoculation on medium), according to the glucidic concentration of the nutritive mediums used in this experiment. We pointed out the maximum lengths of the pollinic tubes, correlated to the lengths of flower stiles from the two taxons. Achieving a certain length of the pollinic tubes is very important, because the fertilization of ovules is thus ensured and, implicitly, the fructification of the respective taxons. We also referred to the way of the emergence of pollinic tubes from the two taxons. The bicellular pollen-type of the two fabaceae is shown by pointing out the generative cell in the pollinic cell, which, after the mitotic division, produces two spermatic cells having the value of immobile male gametes. We have also shown the type of anomalies of the pollinic tubes and the frequency of these anomalies during the process of pollen germination at the two taxons from the Fabaceae family. The obtained results were correlated to the different polyploidity degree of the two taxons, to pollen germinating potential, to geographic spreading area, and to native resistance of the two taxons to various abiotic factors. Although the phylogenetic connection between the two investigated taxons is very tight, there are distinctive traits of the germinating process, which have a diagnosis value and may represent valuable genetic and physiological indicators.

***Key words**: pollen grain, pollinic tubes, anomalies of the pollinic tubes, nutritive medium which sucrose*

The characterization of pollen germinating potential may be fortunately completed by investigations concerning the specific features of the germinating process, therefore by the peculiarities of the male gametophyte development. The male gametophyte develops according to an algorithm specific to species or genus. This process has a direct impact on plant fructification and reproduction. It results the special importance of the pollen as an organ participating in the achievement of one of the most vital stages in the vegetal world - reproduction. Therefore, this paper focuses on the specific aspects of the pollinic germinating process from the two taxons of the *Fabaceae* family: *Trifolium montanum* L. and *T. pannonicum* Jacq. These taxons have different polyploidity degrees, which may be responsive

of the traits of the germinating potential and the cytological characteristics of the germination process. Therefore, *Trifolium montanum* L. is diploid (x = 8; 2n = 2x = 16), while *T. pannonicum* Jacq. is 12 ploid (x = 8; 2n = 12x = 96) [2].

MATERIAL AND METHOD

The biological material consisted in two vegetal taxons sampled from a stationary Tașca- marshalling yard, situated in the neighbouring area of the Ceahlău National Park. This stationary is affected by polluting noxa, which come from the cement factory of Tașca. The two taxons are *Trifolium montanum* L. and *Trifolium pannonicum* Jacq. Pollen was sampled from the two taxons, at the anthesis phase, and was analysed from the viewpoint of the germination process.

Pollen grains have been inoculated on agar nutritive mediums, at which sucrose was added at different crates, from 0 to 350%. The quantity of pollen inoculated on these mediums was the same in all the cases. For each experimental variant, we have done 15 preparations. In order to maintain a wet medium, which was vital for pollen viability, we have used “van Tieghem” wet chambers.

Micromeasurements for determining the dynamics for the extension of pollen tubes were carried out every 3, 24, 48 and 72 hours since the inoculation of pollen grains on nutritive medium. For pointing out the characteristics of pollen tube from this taxon, photographs were taken at Hund Wetzlar optical microscope and drawings at camera lucida.

RESULTS AND DISCUSSIONS

The cytological analysis of the germination process was done at the same time with the analysis of the germinating potential.

After 3 hours since the inoculation of the *Trifolium montanum* pollen on artificial medium, pollinic tubes emerged at different rates, on the variants of nutritive mediums with 0-200% sucrose. Their ratio (2.3-51.7%) [5] is directly correlated to the germinating potential. The mean length of pollinic tubes was comprised between 110 and 199 μm (*fig. 1*). The longest tubes emerged on 25% sucrose medium, resulting in the highest percentage of germinated cells (51.7%) [5].

After the same time interval, the *T. pannonicum* pollen has resulted in pollinic tubes only on four medium variants with 5-50% glucidic composition. In this case, the value of the germinating potential was of 1-20% [5]. The mean length of the pollinic tubes varied between 91 and 255 μm, the maximum values being obtained on 25% sucrose medium (*fig. 1*), directly correlated to the germinating potential.

After 24 hours since inoculation, the number of *T. montanum* pollinic tubes has increased in tight correlation to the maximum germinating potential (4-63%) [5]. In addition, the opportunity of the pollinic tube emergence was also extended on mediums enriched with 300 and 350% glucide. The length of the pollinic tubes reached 116-637 μm (*fig. 2*).

In *T. pannonicum*, after 24 hours, the pollinic tubes emerged on sucrose hyperconcentrated mediums (100-350%). At this taxon, a higher growth was

noticed in the pollinic tubes that reached lengths of 118-779 µm (*fig. 2*). The growth of pollinic tubes is directly correlated with the percentage increase of germinated grains after this time interval (2-51%) [5].

In *Trifolium montanum*, after 48 hours since inoculation, the pollinic tubes increased in length in all the experimental cases, achieving values comprised between 187 and 674 µm (*fig. 3*). This time, the growth of pollinic tubes is in reverse correlation with the germinating potential on 0-50% sucrose mediums (2.5-59% germinated pollen) and in direct correlation with the germinating potential on 100-350% sucrose mediums (31-50% germinated pollen) [5].

In *T. pannonicum*, the elongation of pollinic tubes was significant on all medium variants, reaching values comprised between 253 and 790 µm (*fig. 3*). In this taxon, too, we found the same correlations between the extension of pollinic tubes and the germinating potential, which is diminishing on 1-50% sucrose mediums (5-50% germinated pollen) and increasing on 100-350% sucrose mediums (10-17% germinated pollen) [5].

After 72 hours since inoculation, many pollinic tubes are resorbed. In *Trifolium montanum*, the surveying pollinic tubes show a diminution in length, therefore their edges are of 133 and 571 µm (*fig. 4*). This phenomenon is directly proportional to the germination capacity shown after this time interval (3-50% germinated pollen) [5]. In *T. pannonicum*, the pollinic tubes on 5% sucrose medium degenerate completely. The pollinic tubes are viable on mediums with 15-350% sucrose. At this taxon, too, they found a diminution in the length of pollinic tubes, values varying between 110 and 673 µm (*fig. 4*). In this case, too, the extension of pollinic tubes is positively correlated with the germinating potential after 72 hours since inoculation (4-28%) [5].

The analysis in dynamics of the pollinic tube length showed that mediums enriched with 25 and 50% sucrose were the most adequate for the emergence of the longest pollinic tubes from the two taxons of *Trifolium* genus (*fig. 5, 6*). The pollinic tubes grow significantly after 24 hours since inoculation. Afterwards, the growth continues insignificantly until 48 hours since inoculation, and then the gradual regression appears. After 72 hours since inoculation, the lengths of pollinic tubes from the two taxons have relatively high values, especially on sucrose hyperconcentrated mediums (100-350%).

We noticed that the *Trifolium montanum* pollen could emerge pollinic tubes on mediums poor in glucidic content (0%, 5% sucrose), even after 3 hours since inoculation, while the *T. pannonicum* pollen is not adapted to germinate on such mediums.

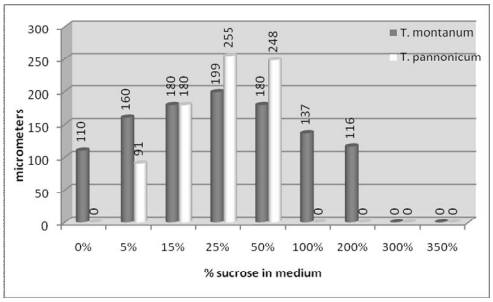

Figure 1 **The average lenght of the pollen tube, 3 hours after inoculation**

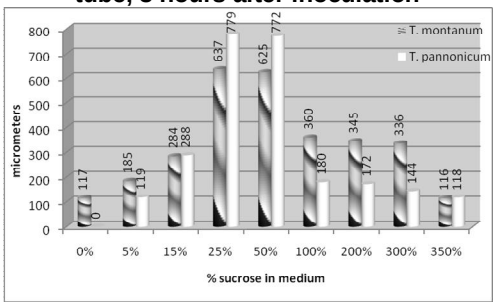

Figure 2 **The average lenght of the pollen tube, 24 hours after inoculation**

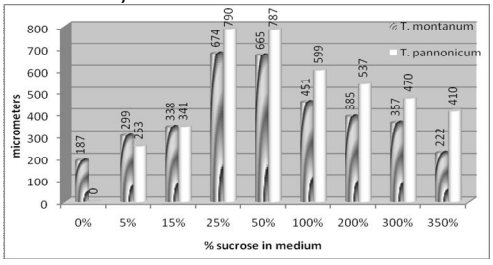

Figure 3 **The average lenght of the pollen tube, 48 hours after inoculation**

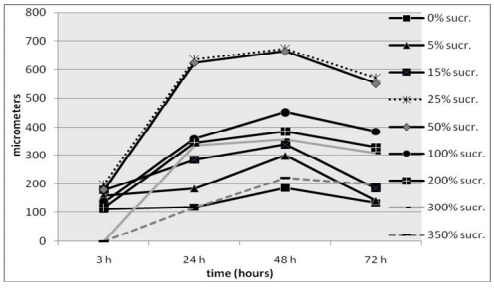

Figure 5 **Dynamics of average lenght of the pollen tube at *Trifolium montanum***

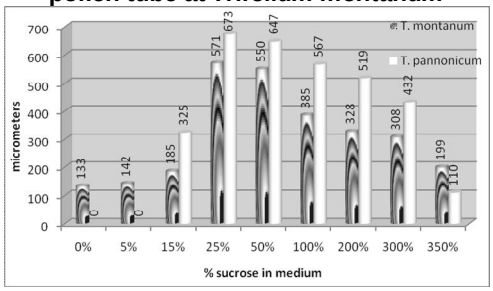

Figure 4 **The average lenght of the pollen tube, 72 hours after inoculation**

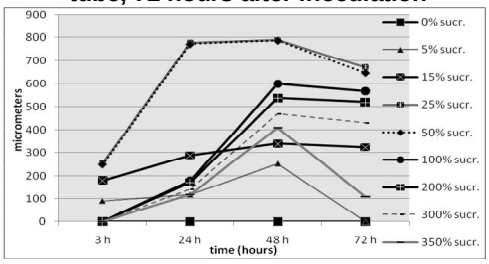

Figure 6 **Dynamics of average lenght of the pollen tube at *Trifolium pannonicum***

The pollinic tubes from *Trifolium pannonicum* are longer as compared to the ones of *T. montanum.* We can explain this phenomenon if we take into account the length of the flower stiles from the two taxons. The *Trifolium pannonicum* flower stile has the mean length of 14 mm, while the same floral element from *T. montanum* has 2.27 mm (original values). Therefore, the flowers and inflorescences of *T. pannonicum* are larger than those of *T. montanum.* There is a direct correlation, genetically determined, between the length of stile and that of pollinic tubes that must penetrate the tissues of the stile to the ovules. It is very likely that "in vivo", the pollinic tubes from the two taxons should be longer than those obtained "in vitro" [4].

There is an easiness with which the pollen from the two *fabaceae* produces pollinic tubes on mediums, which glucidic concentration reaches the oversaturation threshold (100-350%).

The different aspects of *Trifolium montanum* and *T. pannonicum* pollinic tubes, formed on different nutritive mediums are presented in *figures 7- 9.*

Another phenomenon drawing attention is the long-term viability of *Trifolium montanum* pollinic tubes, which, even after 144 hours since inoculation, kept unchanged their vitality even on hyperconcentrated mediums. This remark is not true for *T. pannonicum* pollinic tubes which viability is limited to at most 80 hours.

As concerns the moment of the generative cell division, we found that at both taxons, the generative cell emerges inside the pollinic tube at a great distance from the nucleus of the vegetative cell. Therefore, it results that *T. montanum* and *T. pannonicum* pollen is bicellular, this assessment being conformed to data from literature [3].

The germination process starts by forming a small hill. In 1-2 hours, the small hill is lengthened, and thus the pollinic tube emerges. The nucleus of the vegetative cell is cantoned on the top of the tube, and the generative cell penetrates the tube, being maintained at a certain distance from the vegetative one.

The viable pollinic tubes have a constant diameter on all the length, while the tubes, which are broken on top or near the top, have a descending diameter towards the top. On low and hyperconcentrated sucrose mediums, the inoculated pollen grains have known expulsions of the living content at high rates, especially in *Trifolium pannonicum*.

The pollen germination process of the two *fabaceae* was marked by the emergence of atipic forms of pollinic tubes, known in literature as anomalies [1]. These anomalies consisted in tubes, which were branched at the base or on the top, and in pollen grains with two or three tubes of equal or different lengths. In case of branches or of two or three tubes pollen, the generative cell was cantoned in a single branch, respectively, tube. These anomalies were correlated neither to the length of the pollinic tube or to the glucidic concentration of the artificial medium. These types of anomalies were pointed out at both taxons. But the frequency of anomalies was higher in *Trifolium pannonicum* pollen (23%), as compared to *T. montanum* (11%) (*fig. 10-12*).

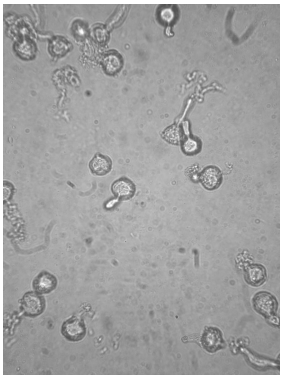

Figure 7 **Pollen germination on 5% sucrose medium, 3 hours after inoculation in *Trifolium montanum* (1000X) (Original)**

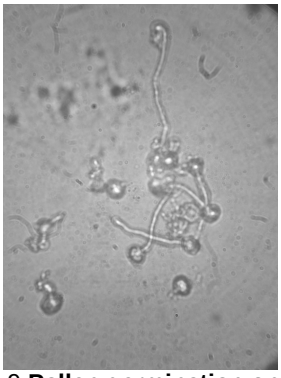

Figure 9 **Pollen germination on 100% sucrose medium, 24 hours after inoculation in *Trifolium montanum* (1000X) (Original)**

Figure 8 **Pollen germination on 25% sucrose medium, 3 hours after inoculation in *Trifolium pannonicum* (400X) (Original)**

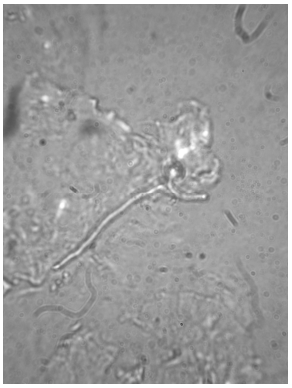

Figure 10 **Pollen tubes branched, in *Trifolium pannonicum* (400X) (Original)**

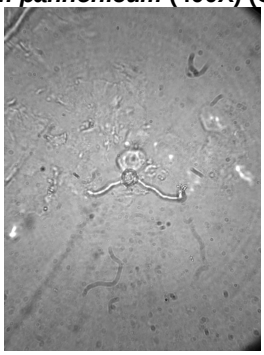

Figure 11 **Pollen grain with two tubes, in *Trifolium montanum* (1000X) (Original)**

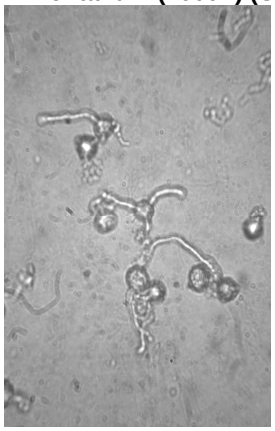

Figure 12 **Pollen grain with two tubes, in *Trifolium pannonicum* (400X) (Original)**

CONCLUSIONS

The generative cell of *Trifolium montanum* and *T. pannonicum* pollen is divided in the pollinic tube.

The longest pollinic tubes in *Trifolium montanum* and *T. pannonicum* may be formed on 25% and 50% sucrose mediums, after 24 and 48 hours since inoculation.

The length of *Trifolium montanum* and *T. pannonicum* pollinic tubes is, generally, positively correlated to the germination potential and especially, to the length of flower stiles. The longevity of *Trifolium montanum* pollinic tubes on mediums with hyperconcentrated glucidic composition is over 144 hours, while that of *T. pannonicum* is at most 80 hours.

During the pollen germination of the two taxons, there occurred anomalies of the pollinic tubes (branched tubes, 2-3 tubes/grain pollen), which frequency is higher in *Trifolium pannonicum.*

The *Trifolium montanum* and *T. pannonicum* pollen has the ability of making pollinic tubes on mediums which glucidic concentration reaches saturation. This demonstrates that the two taxons and, especially *Trifolium montanum,* have a high native resistance at long-term droughts.

The moderate values of the proportion of pollinic tubes, their emergence on a small number of experimental variants, the high frequency of anomalies in pollinic tubes, demonstrate the low fertility degree of *Trifolium pannonicum* pollen. This phenomenon confirms the presence of self-polyploidity (12x) at that taxon.

BIBLIOGRAPHY

1. Büyükkartal, H.N., 2003 - *In Vitro Pollen Germination and Pollen Tube Characteristics in Tetraploid Red Clover (Trifolium pratense L.)*, Turk J. Bot. p. 57-61.
2. Ellison, N.W., Liston, A., Steiner, J.J., Williams, W.M., Taylor, N.L., 2006 - *Molecular phylogenetics of the clover genus (Trifolium-Leguminosae)*, Molecular phylogenetics and evolution, vol. 39, n^0 3, p. 688-705.
3. Hindmarsh, G.J., 1964 - *Gametophyte development in Trifolium pratense L.*, Aust J. Bot., vol. 12, p. 1-14.
4. Kendal, W.A.,1967 - *Growth of red clover pollen. II. Elongation in vitro*, Crop Sci. 7, p. 342-344.
5. Pădureanu, Silvica, 2009 – *Morphological characterization and the germinating potential of Trifolium montanum L. and T. pannonicum Jacq. pollen*, Anale st. Univ. Al.I. Cuza, Iassy, tom LV , fasc. I, s. II a, Biol. veget., p. 107-115.