MORPHOLOGICAL CHARACTERIZATION AND THE GERMINATING POTENTIAL OF TRIFOLIUM MONTANUM L. AND T. PANNONICUM JACQ. POLLEN

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Abstract: The paper presents the general morphological aspects of Trifolium montanum L. and T. pannonicum Jacq. pollen with the diagnosis value of the respective taxa. It also presents the variability of pollen germinating capacity in the two taxons from the fabaceae family, according to the factor of glucidic concentration in the artificial germination environment. We have tested germination in nutritive mediums lacking glucidic elements and in mediums with different sucrose concentrations, until 350%. This paper presents the concentration of glucidic elements from mediums allowing pollen germination at minimum percentage, shows the sucrose concentrations for developing the germination process under best conditions and analyses the germination process in dynamics (after 3, 24, 48 and 72 hours since pollen inoculation in nutritive mediums). The obtained results may be correlated to the amplitude of the variability in pollen grain size, to the ecological plasticity typical of the two taxons, plasticity controlled by the genetic substratum typical of the two tightly related species. It was shown that the pollen germinating potential was different, being a species trait, with diagnosis value. The Trifolium montanum pollen having a higher germinating potential, it could be correlated to the vast spreading area of this species, which is represented in europe, caucasia, siberia, persia, america.the germinating potential of the Trifolium pannonicum pollen (with spreading area in europe and in north of northern of america), lower than of the other taxon, could be explained by the high polyploidy degree.

Key words: pollen grain, exine ornamentation, germinating potential of pollen

Introduction

The study of pollen represents a tool for completing the study of the floral biology of spontaneous and cultivated plants. The knowledge of plant phylogeny is facilitated by the pollen study, as it keeps its shape and structure along the successive generations. Therefore, genera from pre-quaternary ages may be diagnosed according to their fossil pollen. The morphological and structural specific features of the exine are the main unchanged diagnosis traits in all the geological ages [7].

The complex cytopalinological investigations pointed out that pollen morphology has expressed the polyploidity degree of the respective taxon, which determined the variations issued in the morphopalinological characteristics and in the fertility degree [2, 3].

The present study focuses on the morphological specific features and the limits of the pollen germinating potential at two taxons from the spontaneous flora - Trifolium montanum L. and T. pannonicum Jacq., from Fabaceae family. The germinating potential is correlated to some morphological traits of pollen grains [8], for pointing out their importance in defining the pollen fertility degree.

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Material and methods

The biological material is represented by two vegetal taxons taken from a stationary (Tasca-marshalling yard) placed in the surroundings of the Ceahlau National Park. This stationary is affected by polluting noxa, which come from the cement factory of Taşca.

The two taxons are *Trifolium montanum* L. and *Trifolium pannonicum* Jacq.

From the two taxons we took pollen at the anthesis phase. The pollen was studied as concerns the morphological specific features and the germinating potential. In order to define the pollen morphology, we determined shape of pollen grains, exine ornamentation, grain sizes. For determining the shape of pollen grains and of exine ornamentations, we have used the Tesla electron-scan microscope, at which we took microphotographs [4]. For determining the size of pollen grains, we did micromeasurements at 1000 grains/taxon. We measured the longitudinal and the equatorial diameter. The obtained data were used for determining the biostatistical indices [1]: the standard deviation (S), the variation coefficient (S %) and the mean error of the arithmetical mean (Sx). For determining the germinating potential, we have used the so-called van Tieghem “wet rooms”[5]. The nutritive mediums necessary for the germination of pollen grains consisted in distilled water, agar 1% and sucrose at different rates: 0%, 5%, 15%, 25%, 50%, 100%, 200%, 300% and 350%. Thus, nine experimental variants resulted for each taxon. For each experimental variant, we have used 20 “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 3, 24, 48 and 72 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 non-germinated ones. The values from tables represent the arithmetical means of the determinations, which were carried out.

Results and discussions

Shape of pollen grains and exine ornamentations

*Trifolium montanum* L. has ovoid-lengthened grains; the transversal diameter is constant on the entire length of the grain and the exine is thinly reticulated (fig. 1, 2).

*Trifolium pannonicum* Jacq. has elliptic grains and the exine ornamentation is faveolate (it has low depth or faveol depressions) (fig. 3, 4).

Size of pollen grains

Measuring the diameter of pollen grains is one of the safest ways, which separates the diploid plants to self-tetraploid or mixoploid plants. Doubling the number of chromosome sets is correlated to the increase in size of pollen grains and to the increase in ratio of sterile grains.

Because of the lengthen shape of pollen grains from the two taxons, we have determined two diameters: longitudinal and equatorial ones.

The mean value of the longitudinal diameter of *Trifolium montanum* pollen is of 39.537 µm, and that of the equatorial diameter is of 21.545 µm. In *Trifolium pannonicum* pollen, the same parameters have much higher values: the longitudinal diameter is of 59.369 µm and the equatorial one is of 32.344 µm. The ratio between the two diameters is almost identical at both taxons, having values of 1.835 µm and 1.836 µm (tab. I, II). All the
other biostatistical indices (variation amplitude, $S$, $S\%$, $S\bar{X}$) have higher values at *Trifolium pannonicum* pollen (tab. I, II). From the statistical analysis of the pollen size in the two taxons, it results that *Trifolium pannonicum* pollen grains have a higher variability than the ones of *T. montanum*, variability that has its explanation in the polyploidity degree 12x, known in literature at *Trifolium pannonicum* [5].

**Germinating potential**

After 3 hours since inoculation, the *Trifolium montanum* L. pollen can germinate at a rate of 2.3%, on a medium completely lacking glucidic elements, while on mediums at which sucrose is added at rates between 5 and 25%, the ratio of germinated grains increases gradually, reaching the maximum percentage (51.7%) on 25% sucrose medium, then decreases on 50-200% sucrose mediums, when the percent values of germination are of 35-2.7% (fig. 5, tab. III). The *Trifolium pannonicum* pollen does not germinate on mediums lacking glucidic elements. The lower limit at which pollen germination is allowed takes place on 5% sucrose medium, at a rate of only 1%. Higher values of germinability are found on 15-50% sucrose mediums, the maximum rate being expressed on 25% sucrose medium, when 20% grains are germinated (fig. 5, tab. IV).

After 24 hours since inoculation, the percent values of *Trifolium montanum* pollen germinability increase significantly at all the variants, and the palette of sugar concentration, which allows pollen germination, is larger, reaching 350%. After this time interval of inoculation on medium, the highest rate of germinated pollen was also registered on 25% sugar medium. The 50% sucrose medium has also allowed germination at a very close level to the one achieved on 25% sucrose medium (fig. 6, tab. III). In *T. pannonicum*, after 24 hours since inoculation on medium, increases are, generally, found in percent values of pollen germinability, on 5-50% sucrose mediums. After this time interval, germination was also possible on mediums hyperconcentrated in glucidic elements (100-350% sucrose), but at low levels. The maximum rate of germinated pollen was registered on 25% sucrose medium (fig. 6, tab. IV).

After 48 hours since inoculation, in case of *Trifolium montanum* pollen, we found an insignificant diminution in the percentage of germinated grains on 0-200% sucrose mediums, while on 300 and 350% mediums the values have significantly increased. On 25 and 50% sucrose mediums, the maximum rate of germinated pollen is maintained (fig. 7, tab. III). In case of *T. pannonicum* pollen, after 48 hours since inoculation on medium, we found a slightly regression of the percent values on 5-50% sucrose mediums. On 100-350% glucidic substance mediums, an increase was found in the rate of germinated pollen (fig. 7, tab. IV).

After 72 hours since inoculation, the germinating potential of *Trifolium montanum* pollen diminished at all the experimental variants while on sucrose lacking medium, germinability was absent. The highest values of germinability are maintained on 25 and 50% sucrose mediums (fig. 8, tab. III). After this time interval since inoculation, in *T. pannonicum*, too, the germinating potential diminishes in all the experimental cases. On 5% sucrose medium, the germinating process can no longer reveal itself. The maximum rate of germinated pollen after 72 hours since inoculation at this taxon is done on 25% sucrose medium (fig. 8, tab. IV).

If we analyse in dynamics the pollen germinating potential of the two taxons from the *Fabaceae* family, we find that in *Trifolium montanum*, the highest values are achieved on 25 and 50% sucrose mediums, after 24 and 48 hours since inoculation. Under these
conditions, the pollen germinating potential of *Trifolium montanum* is of 59-63% (fig. 9). In case of *T. pannonicum* pollen, the maximum germinating potential is found only on 25% sucrose medium, after 24 and 48 hours since inoculation, with values of 48-51% (fig. 10).

**Conclusions**

The shape of pollen grains from *Trifolium montanum* L. and *Trifolium pannonicum* Jacq. is, generally, prolate, having the value of ratio between the polar axe and the almost identical equatorial axe - 1.83 µm. At a detailed analysis, the pollen of the two taxons has no identical shape. In *Trifolium montanum*, the grains have the same width on the entire length, while in *T. pannonicum*, the equatorial axe is greater than the rest of grain length.

The ornamentation of the exine is different at the two taxons, being thinly reticulated in *Trifolium montanum* and foveolate in *Trifolium pannonicum*.

The pollen from the two taxons also differs by its size. The *Trifolium pannonicum* pollen grains have almost the double size as compared to that of *Trifolium montanum*.

From the size point of view, the pollen grains from *Trifolium pannonicum* are very heterogeneous, having a mean variability degree, which indicates that this taxon is polyploid.

The pollen germinating potential of the two taxons from the *Fabaceae* family differs by the capacity of pollen grains to germinate on mediums with different glucidic concentrations. The *Trifolium montanum* pollen expresses its germination capacity both on mediums, which are not enriched in sucrose, and on hyperconcentrated mediums of 350% sucrose, while in *Trifolium pannonicum* pollen, the germination process is inhibited on glucide lacking medium, but can be present on hyperconcentrated mediums of 350% sucrose. The values of the germination potential of *Trifolium montanum* pollen are higher, compared to those of *Trifolium pannonicum* pollen, in all the experimental cases.

The maximum rate of pollen germinating potential in *Trifolium montanum* is found under conditions of mediums with 25 and 50% sucrose, while in *Trifolium pannonicum*, the highest level of pollen germinability is achieved only on 25% sucrose medium. The values of maximum germinability in *Trifolium montanum* pollen are higher, as compared to those of *Trifolium pannonicum* pollen.

*In vitro*, the maximum levels of the germinating potential are achieved after 24 hours since pollen inoculation on artificial mediums at both taxons. After this time interval, the germinating potential diminishes in most of the experimental cases. An exception is represented by the pollen inoculated on mediums enriched with 100-350% glucide, which shows a reduction in the germination rate, because of high medium viscosity. On these hyperconcentrated mediums, the values of the germinating potential have increased after 48 hours since pollen inoculation. These increases are more significant in *Trifolium montanum* pollen. But, after 72 hours since inoculation, decreases in the values of pollen germination capacity are found on all the artificial mediums.

The ability of pollen from the two taxons of *Trifolium* genus to germinate even at low rates on 350% sucrose mediums shows an extraordinary great adaptability to long-term drought conditions. The *Trifolium montanum* pollen having a higher germinating potential, it could be correlated to the vast spreading area of this species, which is represented in Europe, Caucasia, Siberia, Persia, America [6]. The germinating potential of the *Trifolium*
*pannonicum* pollen (with spreading area in Europe and in north of Northern of America), lower than of the other taxon, could be explained by the high polyploidy degree, phenomenon caused by the great altitude at which this species is adapted.

The similitude between pollen morphological specific features and of the pollen germinating potential is due to the phylogenetic relation between the two taxons, both of them belonging to the same family and genus. The non-identity of the traits of the analysed pollen demonstrates that they are specific, according to the species.

**REFERENCES**


**Explanation of figures:**

Fig. 1 Pollen grain of *Trifolium montanum* L. (400x)
Fig. 2 Pollen grain of *Trifolium montanum* L. (photography electron microscope SEM)
Fig. 3 Pollen grain of *Trifolium pannonicum* Jacq. (400x)
Fig. 4 Pollen grain of *Trifolium pannonicum* Jacq. (photography electron microscope SEM)
### Table I

Variability of pollen grain size in *Trifolium montanum* L.

<table>
<thead>
<tr>
<th>Type of diameter</th>
<th>Mean value (µm)</th>
<th>Minimum value (µm)</th>
<th>Maximum value (µm)</th>
<th>Variation height (µm)</th>
<th>S (µm)</th>
<th>S%</th>
<th>( \overline{S\times} ) (µm)</th>
<th>Rate high diameter/equatorial diameter (µm)</th>
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### Table II

Variability of pollen grain size in *Trifolium pannonicum* Jacq.

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### Table III

The germinating potential of *Trifolium montanum* L. pollen

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### Table IV

The germinating potential of *Trifolium pannonicum* Jacq. pollen

<table>
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<th>No. hours since inoculation</th>
<th>Sucrose concentration (%) of artificial mediums</th>
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<td>0</td>
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<tr>
<td>3 hours</td>
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<td>24 hours</td>
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<td>72 hours</td>
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Fig. 5 Pollen germination after 3 hours since inoculation on medium

Fig. 6 Pollen germination after 24 hours since inoculation on medium

Fig. 7 Pollen germination after 48 hours since inoculation on medium

Fig. 8 Pollen germination after 72 hours since inoculation on medium
Fig. 9 Germination dynamics of *Trifolium montanum* L. pollen

Fig. 10 Germination dynamics of *Trifolium pannonicum* Jacq. pollen