PECULIARITIES OF POLLEN TUBE GROWTH OF *RHODODENDRON RACEMOSUM* FRENCH.

PARTICULARITĂȚILE DE CREȘTERE ALE TUBULUI POLINIC DE *RHODODENDRON RACEMOSUM* FRENCH.

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Abstract: *This paper is an extension of a previous study on the pollen morphology and germination potential of Rhododendron racemosum French. In this study are referred cytological aspects of process of pollen germination at this taxon. Was examined the growth of pollen tube length based on the concentration of sucrose in mediums, over a period of 192 hours after inoculation of pollen. The increase in length of the pollen tubes was in most cases directly proportional to level of the pollen germination capacity, in sense that the longest pollen tubes were formed on nutrient mediums which permit the bigest germination of the pollen tetrade. In this respect was demonstrated that the medium with 25% sucrose ensure the growth of the longest pollen tubes correlated with the style's length of flower from this genotype. Also highlights the formation stages of pollen tube, his longevity, anomalies in male gametophyte development to Rhododendron racemosum.*
Key words*: Rhododendron racemosum, nutritive medium, pollen, pollen tube*

Rezumat: *Lucrarea reprezintă o extensie a unui studiu anterior referitor la morfologia și potențialul germinativ al polenului de Rhododendron racemosum French. În prezentul studiu se face referire asupra aspectelor citologice ale procesului germinativ al polenului. Se analizează creșterea în lungime a tuburilor polinice în funcție de compoziția glucidică a mediilor nutritive în decursul a 192 ore de la inocularea polenului. Creșterea în lungime a tuburilor polinice a fost în majoritatea cazurilor, în relație de directă proporționalitate cu nivelul capacității de germinare a polenului, în sensul că cele mai lungi tuburi polinice s-au format pe mediile nutritive care permit germinarea a cât mai multor tetrade polinice. În acest sens se demonstrează că mediul cu 25% compoziție glucidică asigură creșterea celor mai lungi tuburi polinice în corelație cu lungimea stilului florii de la acest genotip. De asemenea se evidențiază particularitățile edificării tubului polinic, longevitatea și anomaliile în dezvoltarea gametofitului mascul la Rhododendron racemosum.*
Cuvinte cheie*: Rhododendron racemosum, medii nutritive, polen, tub polinic*

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INTRODUCTION

Within the *Rhododendron* genus there are performed a variety of hybridization with the goal of wider biodiversity of this genus, in ornamental purpose. In addition, in *Rhododendron* genus were identified natural hybrids, as are those between *Rhododendron spiciferum* and *R. spinuliferum*, and populations of *R.* × *duclouxii* represent hybrid swarms from backcrossing or selfing of F_1 plants (Yan *et al.*, 2013). This fact demonstrates for the first time the existence of a natural homoploid hybrid in this genus.

In hybridizations there is very important the quality of male parent pollen. In this regard there have been studies on pollen tube growth in rhododendrons. For example, in the cross *Rhododendron impeditum* × *R. williamsianum*, the majority of the tubes do not reach an ovule because the tubes have often thickened tips or, when they continue growth, they start to coil without penetrating into an ovule (Kho and Baër J., 1970).

Hybrid sterility at rhododendrons occurs from other causes, such as the chromosome incompatibilities during the production of sex cells of F1 (Krebs and Leach,1997).

This paper is an extension of the work on palinological characterization and pollen potential germination at *Rhododendron racemosum*. This paper brings in the foreground the behavior of pollen tube at this taxon.

MATERIAL AND METHOD

The biological material is represented by pollen of *Rhododendron racemosum* French. Pollen was sampled at the anthesis phase.

The pollen germination process was determined with hanging drop (Erdman G., 1952). So, pollen grains have been inoculated on agar nutritive mediums (1%), at which sucrose was added at different concentrations: 0%, 5%, 15%, 25%, 40%, 50%, 70%, 100%. Eigh 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.

In parallel with the study of germination capacity, were made micromeasurements for determining the dynamics for the extension of pollen tubes. In this respect, the readings at microscope were done at 4, 24, 48, 72, 96, 168 and 192 hours since the inoculation of the pollen grains on nutritive medium.

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 Hund Wetzlar optical microscope.

RESULTS AND DISCUSSIONS

Results related to the average length of pollen tubes of *Rhododendron racemosum* depending on the sugar composition of the nutrient mediums and the time from inoculation pollen on mediums are summarized in table 1.

Table 1

The average length of the pollen tube (µm) at *Rhododendron racemosum*

time from inoculation (hours)	% sucrose in medium							
	0%	5%	15%	25%	40%	50%	70%	100%
after 4 h	0	0	0	0	0	0	0	0
after 24 h	345	658	688	855	242	9	0	0
after 48 h	0	1280	1309	1402	1112	756	138	0
after 72 h	0	2268	2295	2359	1123	1110	1106	0
after 96 h	0	2707	2950	3577	1803	1952	1638	0
after 168 h	0	3560	3910	4036	2175	2087	1999	0
after 192 h	0	4144	4200	4385	2995	2767	2520	0

After the first 4 hours after inoculation of the nutrient mediums, pollen tubes no were formed at all in experiment.

After 24 hours since inoculation of the pollen on artificial mediums, occured the first pollen tubes on six mediums: pure water (0% sucrose), 5%, 15%, 25%, 40% and 50% sucrose. The length of pollen tubes formed first varied widely, depending on the sugar composition. So, the shortest were those edified on medium enriched with 50% sucrose, and the longest on medium with 25% sucrose. On the medium very enriched with sucrose, germination has not occurred and therefore have not formed any pollen tubes.

After the 48 hours of the inoculation, the pollen tubes have been stretched enough, were doubling the length in some cases. Pollen tubes formed on deficiencient medium in sucrose, 24 hours ago, were resorbed, completely degenerated. On the mediums with the addition of 70% sucrose, they were built first tubules with an average length of 138 µm. The longer pollen tubes grew on mediums by 5%, 15% and 25% sucrose, the average values were 1280, 1309 and 1402 µm respectively.

After 72 hours, reveals significant increases in pollen tubes already formed. The medium saturated in sucrose (100% sucrose), did not allow pollen tube formation. Also, lack of sugar in medium (0% sucrose) still remains averse for the development of the male gametophyte from this taxa.

After 96 hours of inoculation pollen on artificial mediums, were significant increases in the length of pollen tubes already formed. The longest tubes measured in average 3577 µm, and the shortest 1638 µm. The longest were formed on medium with 25% sucrose, and the shorter on medium with 70% sucrose.

After 168 hours of starting the experiment, pollen tubes already formed in all previous intervals had significant increases. The longest tubes were the conditions on medium with 25% sucrose, followed in descending order by pollen tubes formed in environments enriched with 15%, 5%, 40%, 50%, 70%.

After 192 hours, recorded insignificants increase of the pollen tubes.

Even after eight days after the inoculation, the pollen of *R. racemosum* did not germinate on medium absent in sucrose and on medium with 100% sucrose.

Dynamic analysis shows that the pollen tube growth during 8 days is generally upward. Growth rate is increasing after 48 hours, the maximum rate is on medium with 25% sucrose, when was the longest tubes formed after 8 days (fig. 1).

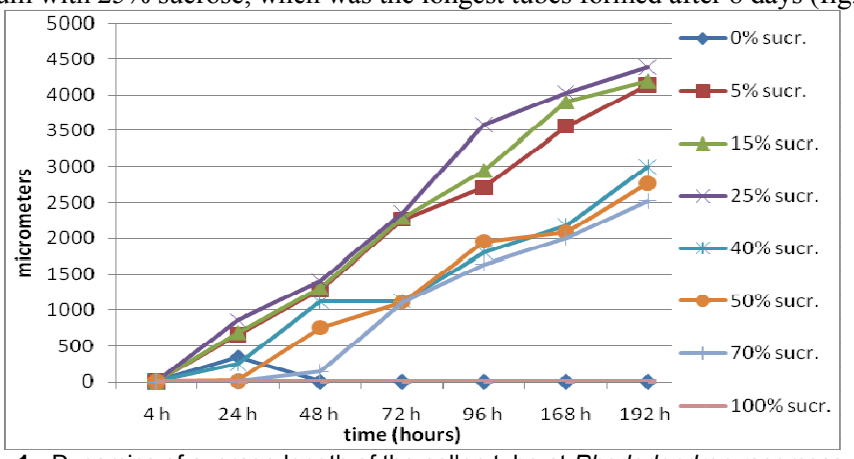

Fig. 1 - Dynamics of average length of the pollen tube at *Rhododendron racemossum*

Analysis of pollen tube growth in length highlights that to *R. racemosum*, the pollen germinated has high viability proven by the viability of pollen tubes that even after 8 days of their growth, they have not degenerated. These findings are consistent with data Palser's *et al*. (1992), according to which, tubes reached the ovary in 5-10 days depending on the species of *Rhododendron* and took several days after entering the upper ovary to reach the base of the placentae. To *R. macgregoriae*, fertilization occurs about 6–7 days after pollination (Williams *et al*., 1991).

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 styles of *R. racemosum* has an average length (obtained by measuring of 100 styles) of 4.75 mm. So, only the pollen tube of at least 4750 µm length can reach to the embryo sac for to make the double fertilization. We estimate that this pollen tube length of *R. racemosum* shall be in natural conditions. Always, *in vitro* the pollen tube rate of growth is much slower (Erdman, 1952). According to Okonkwo. and Campbell (1990) the tubes of *R. racemosum* x *R. barbatum* and its reciprocal covered 100% of the style length in 96 hours, the same as of the intraspecific compatible cross *R. racemosum* x *R. racemosum.* According to Williams and Rouse. (1990), the pollen tube growth rates were generally greater for species of *Rhododendron* with longer pistils and larger pollen. The same correlation is accentuated by Fernández *et al.* (2009) showing that the relationship between pollen size and pistil length among species

suggests that the pollination system may be of less importance as a selective force than flower functionality.

The pollen germination process of *R. racemosum* was marked by anomalies that consisted in dilatations or ramifications at the top of the pollen tube (fig. 2) and in tetrads which 2-3 pollen tubes (fig. 2, 3). In this case the 2-3 tubes do not grow enough and so are not effective in fertilizing. These anomalies are not correlated with sucrose concentration in mediums.

Fig. 2 - Pollen tube with dilatations at tops (left) (100x); branched pollen tube at top (middle) (400x); tetrad with two pollen tubes (right) (100X) (Original)

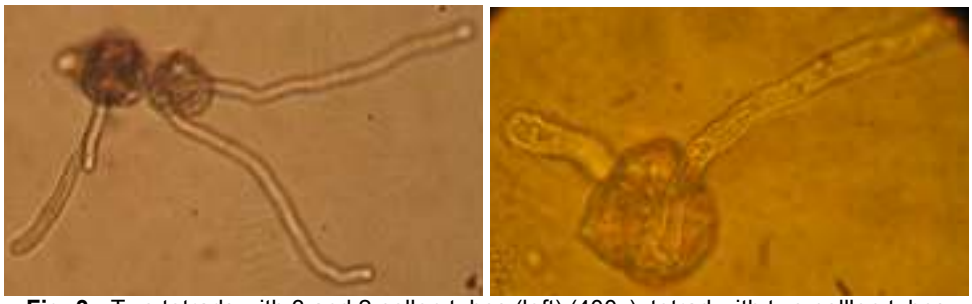

Fig. 3 - Two tetrads with 3 and 2 pollen tubes (left) (400x); tetrad with two polllen tubes (1000x) (Original)

In 4 and 5 photomicrographs are presented aspects of the germination at *R. racemosum.* The germination process at this genotype not starts by forming a vesicle (fig. 4).

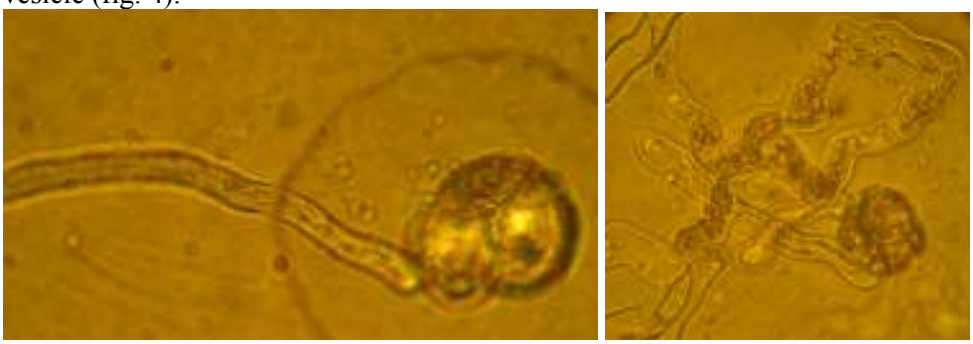

Fig. 4 - The debut of edification of the pollen tube at *Rhododendron racemosum* (left) (1000x); pollen tube after 48 hours since inoculation on medium with 15% sucrose (right) (1000x) (Original)

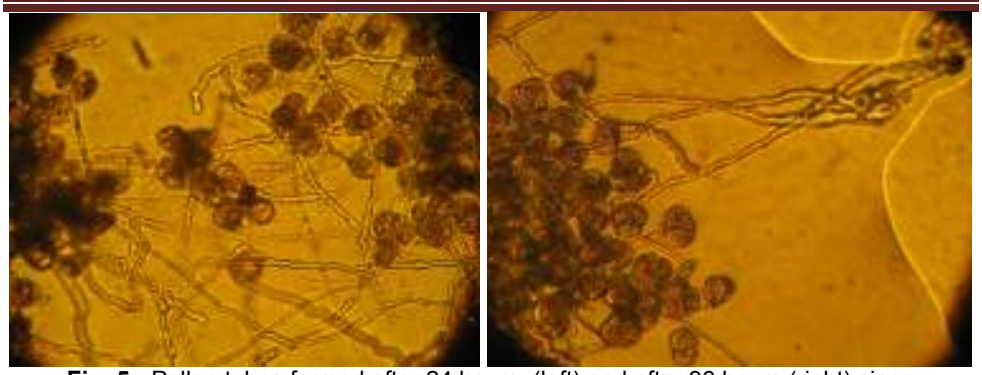

Fig. 5 - Pollen tubes formed after 24 hours (left) and after 96 hours (right) since inoculation on medium with 25% sucrose (400x) (Original)

CONCLUSIONS

The longer pollen tubes more than 4000 μm were developed after 8 days on medium with 25% sucrose. The increase in the length of pollen tubes is directly proportional to the ability of pollen germination. The increase in the length of pollen tubes is in close correlation with the length of floral style; in this case only long pollen tubes can cross over 4000 μm stylar tissue. Extrapolating the *in vivo* situation, the pollen of *Rhododendron racemosum* germinates on a stigmatic liquid containing 25% carbohydrates, and pollen tubes cross the style in about 7 days to reach the ovules and fertilize them.

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