THE PALYNOLOGICAL CHARACTERIZATION AND THE POLLEN POTENTIAL GERMINATION OF *RHODODENDRON RACEMOSUM* FRENCH.

CARACTERIZAREA PALINOLOGICĂ ȘI A POTENȚIALULUI GERMINATIV AL POLENULUI DE *RHODODENDRON RACEMOSUM* FRENCH.

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Abstract. *The palinological characterization of Rhododendron racemosum highlights the organization of pollen in the pollen tetrade maintained by viscin threads. The set of determinations related to pollen morphology (the color of pollen grains, the ornamentation of exin, the pollen tetrad size, the number of pore/pollen grain) from Rhododendron racemosum complements the information in the literature regarding the characterization of from this taxon. To test the germination potential were used many variations of nutrient medium with different concentrations in sugar from 0% to 100%. The pollen germination of Rhododendron racemosum is expressed in the highest degree on the nutrient medium enriched with 25% sucrose, 168 hours after inoculation. Very high germination capacity, over 90% to Rhododendron racemosum pollen demonstrates that the pollen meiosis in this genotype is balanced and the male gametes are fertile. The data obtained have theoretical importance (in taxonomy) and in getting interspecific hybrids within the genus Rhododendron.*
Key words*: Rhododendron racemosum, pollen tetrad pollen, nutritive medium, germination capacity*

Rezumat *Caracterizarea palinologică la Rhododendron racemosum scoate în evidență modul de organizare a polenului în tetrade polinice, menținute prin fibre de viscină. Setul de determinări privitoare la morfologia polenului (culoarea granulelor, ornamentația exinei, dimensiunea tetradelor polinice, numărul porilor germinativi/granulă de polen) la Rhododendron racemosum completează informațiile din literatură referitoare la caracterizarea polenului acestui taxon. Pentru testarea potențialului germinativ s-au folosit mai multe variante de mediu nutritiv cu diferite concentrații glucidice, cuprinse între limitele 0% și 100%. Germinarea polenului de Rhododendron racemosum se exprimă în cel mai înalt grad pe substrat nutritiv îmbogățit cu 25% zaharoză, după 168 de ore de la inoculare. Capacitatea de germinare foarte ridicată, de peste 90% al polenului de Rhododendron racemosum demonstrează că meioza polinică la acest genotip este echilibrată, iar gameții masculini sunt fertili. Datele obținute au importanță teoretică (în taxonomie), dar și practică pentru lucrările de ameliorare pentru obținerea hibrizilor interspecifici din cadrul genului Rhododendron.*
Cuvinte cheie: *Rhododendron racemosum, tetradă polinică, mediu nutritiv, capacitate de germinare*

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INTRODUCTION

The genus *Rhododendron* L., one of the largest and diverse genera of *Ericaceae* comprises over 1000 species. The centre of diversity of the genus is in the Himalaya (Maurizio and Grafl 1969, Podbielkowski 1991, cited by Weryszko-Chmielewska and Chwil M., 2005).

These plants are represented by shrubs of great beauty and elegance with a large spread. The species and cultivars obtained by hybridization are in large numbers. Called "kings ornamental shrubs" around the rhododendrons has developed an entire horticultural specialized on production and marketing of ornamental woody plants.

In the works hybridization has great importance the pollen of parental plants. This study aimed to determine the morphological and germination capacity of pollen from *Rhododendron racemosum* French.

MATERIAL AND METHOD

The biological material is represented by the fresh pollen of *Rhododendron racemosum* French. In order to define the pollen morphology, we determined shape of pollen grains, exine sculpturing, size of pollen tetrad and number of germinative pores/pollen grain. For determining the shape of pollen grains, the apertures (number and arrangement), the exine sculpturing, we have used the Hund Wetzlar microscope, at which we took microphotographs. For determining the size of pollen we did micromeasurements at 1000 pollen tetrads (Oldfield,1959). 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 tetrads. The method consisted in introducing the pollens in a mixture of sulphuric acid and acetic acid. For study of the germination pollen, we have used the hanging drop method (Stanley and Linskens,1985). The nutritive mediums necessary for the germination of pollens consisted in distilled water, agar 1% and sucrose at different concentrations: 0%, 5%, 15%, 25%, 40%, 50%, 70% and 100%. Thus, 8 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 4, 24, 48, 72, 96, 168 and 192 hours since the pollen inoculation in mediums, thus, being established the percent dynamics of the germination capacity for this genoptype. The germination capacity was expressed as percentage, by reporting the number of germinated grains to total pollen grains.

RESULTS AND DISCUSSIONS

The palynological characterization of *Rhododendron racemosum*

The pollen of *Rhododendron racemosum* is spheroidal, white isabelline. The surface sculpturing is psilate.

The pollen grains of this taxa remain adherent in tetrahedral tetrads of four pollen grains. Each tetrad are provided on her outer surfaces with long viscin threads. Viscin threads occur among the pollen tetrads and presumably play a role

in pollen removal from the anthers and its adhesion to pollinators (Sarwar and Takahashi, 2013). Hesse M. *et al.* (2000) suggest that any pollen with viscin threads to the highly specialized pollination mode. According to Hesse M. *et al.* (2000) that viscin threads increase the efficiency of pollination, and their presence implies highly specific pollinators for accurate delivery of pollen to stigma. In our view, pollen in tetrade tetrahedral organization associated with viscina is minimum energy and maximum efficiency.

That pollen from *Ericaceae* and *Onagraceae* presents viscin threads (Hesse M.,1983). In addition, in *Boraginaceae* and *Scrophulariaceae* are reported (Hesse M *et al*., 2000). For taxonomic purpose, the pollen of *Rhododendron* has also been studied by others (Bowers C, 1930; Tarnavschi I. *et al*., 1987; Weryszko-Chmielewska and Chwil, 2005; Zhang *et al*., 2009).

Each pollen grain/tetrad has three equally spaced apertures, having 3-colporate (fig. 1).

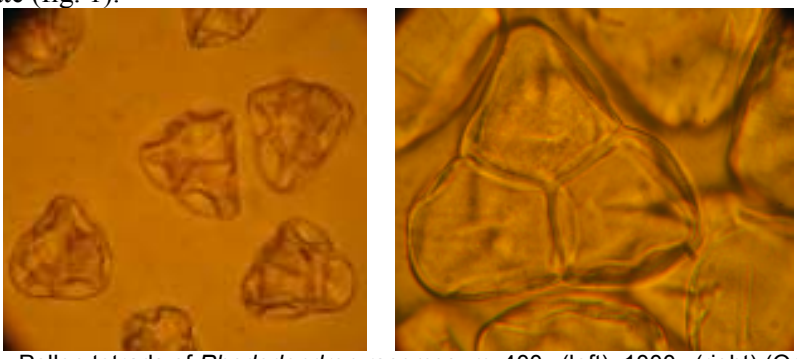

Fig. 1 - Pollen tetrads of *Rhododendron racemosum*: 400x (left); 1000x (right) (Original)

The measurements of pollen tetrads were made in conformance with the recommendations by Oldfield F. (1959), by determining only one side of the tetrad, since all other sides are perfectly equal. Side of tetrad has an average value of 62.79 µm (tab. 1). The coefficient of variation (s%) indicates a low variability for pollen tetrad of *Rhododendron racemosum*. Each pollen grains/tetrad is a tricolporate grain, with the pore area of adjacent grains in contact.

The pollen grains present in the pollen tetrad of *R. racemosum* have more frequently in three pores, rarely two pores or one pore germinating (tab. 2, fig. 2). The data are consistent with the literature (Oldfield, 1959; Tarnavschi *et al*., 1987).

Table 1

Variability of pollen tetrad size in Rhododendron racemosum

Type of axis	Mean value (µm)	Minimum value (µm)	Maximum value (µm)	Variation height (µm)	S (µm)	S%	$S_{\bar{x}}$ (µm)
side of pollen tetrad	62.79	55.20	79.35	24.15	4.83	7.69	0.76

Table 2

Number of germinative pores/pollen grain in Rhododendron racemosum

mean value ($\overline{X}$)	% pollen grains with 1 or 2, or 3 germinative pores		
	1	2	3
2.84	3	10	87

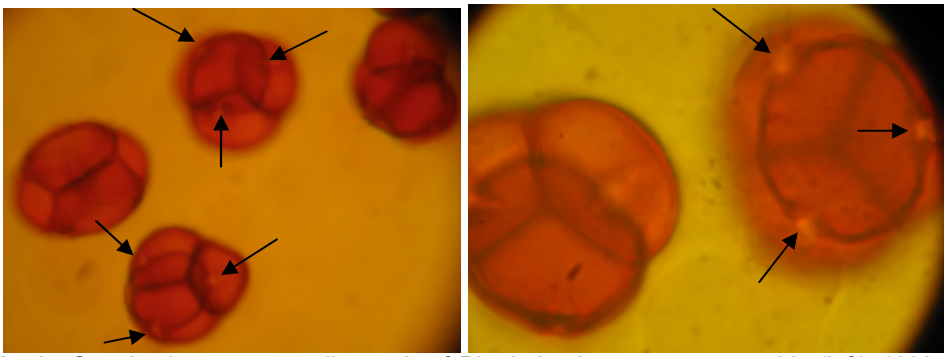

Fig. 2 - Germinative pores at pollen grain of *Rhododendron racemosum*: 400x (left); 1000x (right) (Original) (the arrows indicate the germinative pores)

The fact that the pollen not shows more than 3 germinative pores indicated that probably polyploidy not occurred in this taxa.

Germinating potential of pollen at *Rhododendron racemosum*

Results related to pollen germination of *R. racemosum* are summarized in table 3. Of the four pollen grains of the tetrad, only one germinate. After 4 hours since inoculation of the pollen on nutrient mediums, it was found that no germination were recorded on any of the eight types of medium. After 24 hours of inoculation, pollen germinated on six mediums. On the medium without sucrose and on that with 50% sucrose germinated only 4% and 5% respectively pollen tetrads. On mediums enriched with 25% and 40% sucrose, registered 73% and 72% respectively germinated pollen. After 48 hours, on medium deficiency in sucrose, pollen tubes were resorbed. On the medium with 100% sucrose not germinated pollen at all. Note that three mediums ensured the highest % of germinated pollen:15%, 25% and 40% addition sucrose. After 72 hours, is found that the two mediums (pure water and 100% sucrose) are recalcitrant for pollen germination process. The level increased, maintains in three cases: with 15%, 25%, 40% sucrose, that supported the highest in percentage of germination capacity of the pollen. After 96 hours, the germination potential increases on mediums with 5-70% sucrose. Even after this time, the highest values of the germination capacity are provided from mediums with 15%, 25% , 40% sucrose.
Of these, on medium with 25% sucrose, pollen recorded the highest values of germination.

After 168 hours it is noted that the pollen germination rate values remain almost constant with slight growth (for mediums with 25% to 50% sucrose) comparing to previous interval. On the medium enriched with 25% sucrose, pollen achieved the highest share of the germination (94%). After 192 hours, the germination remains the same as in the previous interval. Even after 8, days the pollen germination was not produced on the mediums with 0% and 100% sucrose.

Table 3

The pollen germination (%) of 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	4	12	32	73	72	5	0	0
after 48 h	0	40	82	84	80	45	19	0
after 72 h	0	70	85	92	85	67	37	0
after 96 h	0	80	85	92	87	77	40	0
after 168 h	0	80	85	94	87	78	40	0
after 192 h	0	80	85	94	87	78	40	0

The dynamic analysis of pollen germination rate of *Rhododendron racemosum* show that in the first 24 hours after inoculation, the pollen germinates explosive on certain mediums. The values of the germination capacity are very different depending on the sugar concentration in mediums. After 168 hours of inoculation reach the maximum values of the germination on medium fortified with 25% sucrose (fig. 3).

Investigations of the potential germination reveal that the viability of pollen of *R. racemosum* it is high. This is in agreement with the literature according to which *Rhododendron* breeders store the pollen up to 2 weeks under the conditions of the special (Widrlechner, 1986).

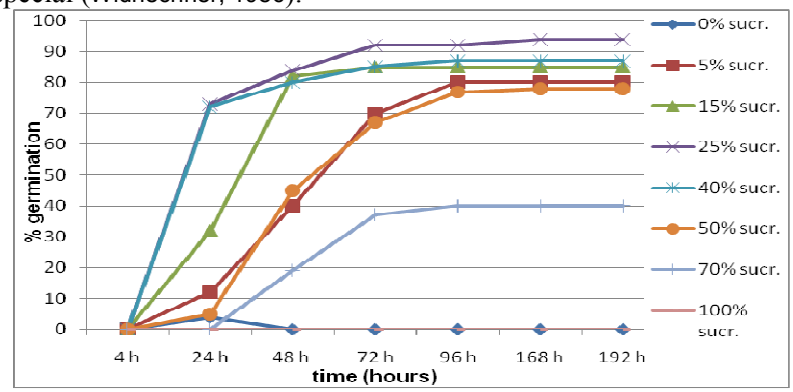

Fig. 3 - The germination dynamics of pollen in *Rhododendron racemosum*

CONCLUSIONS

Pollen of *Rhododendron racemosum* is organized in tetrahedral tetrads, maintained by viscin threads. The pollen grains is tricolporate. The size of pollen tetrads is homogeneous, correlated with high fertility. The maximum values of potential germination achieves after 168 hours after inoculation of pollen on nutrient medium with 25%. The pollen of *R. racemosum* is viable for at least 8 days tested in this experiment, at room temperature.

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