STUDIES REGARDING POLLEN VIABILITY AND GERMINATION CAPACITY OF SOME *VITIS VINIFERA* L. VARIETIES

STUDII PRIVIND VIABILITATEA ŞI CAPACITATEA DE GERMINARE A POLENULUI UNOR SOIURI *VITIS VINIFERA L.*

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Abstract. *Pollen quality is an important indicator in the estimation of grape production, being analysed based on its germination capacity. Thus, viability and germination potential of pollen from six Vitis vinifera L. varieties, grown in the climatic area of the Iasi vineyard, were analysed. To perform the determinations, were used comparatively three methods for observing the viable cells, by treating them with tetrazolium chloride (TTC), Lugol solution (IKI) and methylene blue (AM) solution. The germinating potential was analysed in vitro, the culture being performed on agar medium with added sucrose (0 to 20%). The viable cells were more clearly highlighted using the AM method, but the TTC method was more accurate in indicating the percentage of pollen viability. The highest pollen germination rate was observed in the 15% added sucrose variant.*
Key words: *Vitis vinifera* L., pollen viability, germination capacity

Rezumat. *Calitatea polenului este un indicator important în estimarea producției de struguri, fiind analizată pe baza capacității de germinare a acestuia. Astfel, au fost analizate viabilitatea şi potențialul germinativ al polenului provenit de la şase soiuri de viță de vie (Vitis vinifera L.) cultivate în arealul climatic al podgoriei Iaşi. Pentru efectuarea determinărilorau fost utilizate comparativ trei metode de evidențiere a celulelor viabile, prin tratarea acestora cuclorură de tetrazolium (TTC), soluție Lugol (IKI) şi soluție de albastru de metilen (AM). Potențialul germinativ a fost analizat in vitro, cultura fiind realizată pe mediu de agar cu adaos de sucroză (0 - 20%). Celulele viabile au fost cel mai clar evidențiate cu ajutorul metodei cu AM, metoda cu TTC fiind însă mai precisă în indicarea procentului de viabilitate a polenului analizat. Cea mai ridicată rată de germinare a polenului a fost observată la varianta cu adaos de 15% sucroză.*
Cuvinte cheie: *Vitis vinifera L.*, viabilitatea polenului, capacitate germinativă

INTRODUCTION

The quality of pollen is mainly represented by itsviability and germination capacity, being an essential characteristic that parental plants must accomplish to

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be used in vine-breeding experiments. In the breeding programs, only the descendants with normal hermaphrodite flowers, which allow the vine growing in monovarietalplots, are retained for promotion in production (Oprea and Moldovan, 2007). In the same time, pollen germination capacity is indicated by the potential of the pollen tubedevelopment and its vigor, as essential characteristics for optimal fecundation (Davarynejad *et. al*., 2008).

In vitro tests to determinate the viability and germination capacity of the pollen, indicate the percentage of viable cells, the pollen germination rate and the length of the pollen tube.

Present study aimedto analyse the pollen quality of some *Vitis vinifera* L. varieties for wine and table grapes by testing the viability of the pollen and its germination capacity. Comparative methods to highlight the pollen viability were also tested.

MATERIALAND METHOD

Determinations were performed on six *Vitis vinifera* L. varieties for wine (Chardonnay, Merlot and Cinsaut) and table grapes (Bicane, Muscat de Hamburg and Victoria), growing in the Ampelographic collection of the Research Development Station for Viticulture and Winemaking Iasi, in the years 2015 and 2016.For each variety, 20 inflorescences from 10 normally developed and healthy stocks were harvested randomly, before corollas opening.Inflorescences were placed in dry parchment bags and transported inthe laboratory.The corollas were removed, the anthersbeing isolated and stored overnight at room temperature (25 °C) for a better collection of pollen grains through brushing and sieving.

Pollen viability was estimated using three comparative methods.The tetrazolium chloride method (TTC 1%) is based on the reaction of reduction through respiration of 2,3,5-triphenyl-tetrazolium chloride to red triphenylformazan. The red and pink cells werecounted as viable, microscopic observations being performedaftertwo hours from thecontact of pollen with the solution (Sulusoglu and Cavasoglu, 2014).

When using Lugol's aqueous solution (IKI), viable cells were identified by changing their colour in brown and black, while using methylene blue solution (AM), viable cells remain unstained.Counting viable pollen cells was performed microscopically, after10 minutes fromstaining (Firmage and Dafni, 2001).

To determine the pollen germination capacity (microscopic examination of surfaces with about 100 pollen grains), was used a germination substrate consisting of: 1% agar in distilled water, boric acid (5 mg/L)and sucrose in concentrations of 5, 10, 15 and 20% (pH 6.5; in dark at 30 °C). Pollen wasconsidered germinated when the pollen tube exceeded the length of the pollen grain.

The results are presented as the meanvalues of two years determinations. Analysis ofvariance (ANOVA - Microsoft Excel) was used to investigate the differences between tested methods. P values lower than 0.05 ($p<0.05$) were considered statistically significant. For data dispersion analysis was calculated the coefficient of variation (± / average%).

RESULTSAND DISCUSSIONS

The highest percentage of viable pollen was observed when TTC reagent was used, the values ranging between 82.23 ± 1.31% (Muscat de Hamburg) and 88.34 ± 1.10% (Victoria), with a mean value of 84.87% (tab. 1).

The experimental results indicated statistically significant differences between the methods for testing the pollen viability at all studied varieties. Regardless of the test method used, Muscat of Hamburg showed the lowest percentage of fertile pollen. Also, the lowest pollen viability was obtained when the IKI solutionwas used.

Table 1

Pollen viability of *Vitis vinifera* L. analysed varieties (%)

Genotype/ Method	TTC	IKI	AM	Signification (p<0.05)
Muscatde Hamburg	82.23±1.31	59.79±2.34	75.49±0.89	***
Victoria	88.34±1.10	74.11±1.74	81.28±4.64	***
Bicane	86.44±1.27	71.18±1.50	82.44±3.15	***
Chardonnay	83.50±1.02	61.66±1.73	80.26±1.50	***
Merlot	85.51±1.82	70.34±0.78	84.33±1.57	***
Cinsaut	83.23±1.26	65.71±1.03	77.60±1.78	***
Mean	84.87±2.30	67.13±5.68	80.23±3.23	***
CV%	2.71	8.46	4.03	-

Note:TTC - tetrazolium chloride; IKI -Lugol solution; AM -methyl blue; ± - standarddeviation(between the two years of study); CV% - coefficient of variability.*** - very significant differences (between tested methods).

It was noticed the high variability between varieties of the results obtained with the IKI method, the coefficient of variability (CV%) exceeding 8%.

The concentration of sucrose in the germination media had a significant influence on pollen germination ($p < 0.05$), the germination rate exceeding 80% only for the media with 15% sucrose (tab. 2).

Table2

Pollen germination rates of *Vitis vinifera* L. analysed varieties

Genotypes	Sucrose concentration				Significance (p < 0.05)
	5%	10%	15%	20%	
Muscat de Hamburg	59.35±1.27	61.27±1.33	82.73±2.13	77.88±0.77	***
Victoria	61.44±2.52	69.54±1.25	88.83±0.25	72.38±1.63	***
Bicane	55.37±1.51	62.22±1.42	81.33±1.12	68.33±1.11	***
Chardonnay	61.21±1.71	65.56±1.89	83.42±1.70	70.33±2.70	***
Merlot	62.16±2.90	64.11±1.75	88.05±0.23	74.74±1.05	***
Cinsaut	59.04±3.05	63.39±1.34	89.63±1.29	68.12±1.11	***
Average	59.76±2.48	64.35±2.94	85.66±3.57	71.96±3.83	***
CV%	4.14	4.58	4.17	5.33	-

Note: ± - standard deviation (between the two years of study); CV% - coefficient of variability.*** - very significant differences($p < 0,05$)(between the germination rate on media with different concentrations of sucrose).

The results obtained are in accordance with the data presented by Sabir (2015), regarding the germination rateofpollen at *Vitis vinifera* L. varieties.

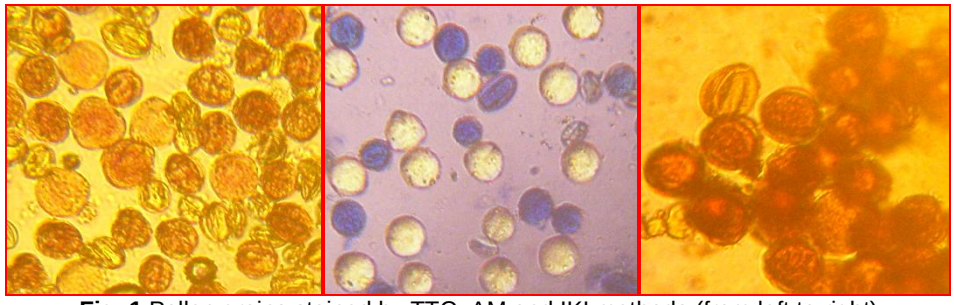

Fig. 1 Pollen grains stained by TTC, AM and IKI methods (from left to right)

The method that differentiated best the viable and non-viable cells was the AM test, followed by the TTC and the staining of the cells with IKI (fig. 1).

CONCLUSIONS

1. For all *Vitis vinifera* L. analysed varieties, the highest percentages of viable pollen were recorded when the TTC method was used, Muscat de Hamburg variety showing the lowest percentage of viable pollen regardless of the tested method.

2. The use of a medium with 15% sucrose led to higher ratesof pollen germination, up to 80% for all analysedvarieties, above the limit of 30% considered necessary for grapevine hybridization.

3. The method that highlighted best the viable cells was the AM method, the TTC method being considered the most accurate in indicating the percentage of viable pollen, in comparison to the germination rate.

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