RESEARCHES REGARDING SOME FEATURES OF APPLE POLLEN TUBES

CERCETĂRI PRIVIND UNELE PARTICULARITĂȚI DE DEZVOLTARE ALE TUBULUI POLINIC LA MĂR

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Abstract*. The pollen tubes are not always appropriate in pistil penetrating. The object of this work is to present two types of "features" that occur during the pollen germination process in vitro at some apple varieties like Pionier and Generos regarding the development and the pollen tube aspect in experimental conditions on different culture media. The manifestation of these "features" was observed on other additional experiments of apple varieties which suggests that it is an "anomaly germination", especially dependent on the species and it is not just a characteristic of the variety own. The microscopic examination of pollen germination analysis was performed by transmitted light and in phase contrast for highlighting of the pollen tubes and pollen nucleus. This kind of germination was observed in three replicates at the rate of 1 to 5%.*
Key words*: pollen tubes, anomaly germination.*

Rezumat*. Tuburile polinice nu sunt totdeauna adecvate străbaterii pistilului. Scopul acestei lucrări este de a prezenta doua tipuri de "particularităţi" care apar în timpul procesului de germinare al polenului in vitro la unele soiuri de măr precum Pionier şi Generos, privind dezvoltarea şi aspectul tubului polinic în condiţii experimentale pe medii diferite de cultură. Manifestarea acestor "particularităţi" a fost observată şi la alte soiuri de măr experimentate suplimentar, ceea ce ne sugerează că este o "anomalie de germinare" dependentă mai ales de specie şi nu este doar o caracteristică proprie a soiului. Examenul microscopic pentru analiza germinării polenului s-a executat în lumină transmisă şi în contrast de fază pentru punerea în evidenţă a tubului polinic cu cei doi nuclei. Acest fel de germinare s-a observat în procent de 1 şi 5%.*
Cuvinte cheie*: tuburi polinice, anomalie de germinare.*

INTRODUCTION

In the present research, it was experimented *in vitro,* the capacity of the pollen germination at two apple varieties namely Pionier and Generos that have different repening times. The age of trees varies from 7 to 10 years with medium and late repening period depending on variety (for Pionier, the beginning of September and the end of September and the beginning of Octomber for Generos variety). The natural fertility, vitality and ability of pollen germination *in vivo* (as a biological feature) have major implications in the quality and quantity of fruits

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production. The experiments of laboratory of freshly pollen germination, released from anthers *in vitro* , are a good test for early assessment and forecasting of the flower fertility rates *in vivo* in the fruit trees orechard (Hedhly *et.al,.* 2004). The pollen germination is a different phase preceding of the development phase of the pollen tube in styl. Each of the two stages (germinations and pollen tube growth) are stimulated by other heating conditions such as: (a) moderate temperature which stimulates the secretions of stigma, increases the pollen adhesion and promotes the germination (Hedhly *et.al.,* 2004); (b) the slightly higher temperature that accelerates the growth pollen tube and stimulates the fertilization process (Hedhly *et.al.*, 2004). Other authors (Pădureanu, 2007) have analyzed in other species as *Vitis vinifera* and *Ampelopsis*, some abnormal forms development of pollen tubes during germination, abnormalities correlated with important aspects of genetic and physiological nature of respective taxa during the germination. In another scientific paper, the same author refers to abnormal pollen tubes which may occur in *Lotus corniculatus* during germination (Pădureanu, 2007). The objective of this research is analyzed by microscopic methodes of the pollen tubes that develops during the germination process and by the evidence of some specific defects that may occur during their development/deployment.

MATERIAL AND METHOD

The samples consisted of open flowers (anthesis) and flowers buds were collected for each variety from 10 trees from both the edge and inside the plot from the orchard. To estimate the germination capacity of pollen the anthers were easily detached from the stamen filament with a sterile needle, there put in a small watch glass with a few drops of distilled water to stimulate the release of the pollen from the anthers, thus making also the pollen hydration. Acording to the experimental results from previuos years we opted for the planting media only with pollen hidrated beforehand. Pollen previously hydrated every watch glass constituted or represented average sample for each variety being assessed. There were used extracted anthers from many flowers, to have a homogeneus sample that represent as closely a biological potential of the pollen at that time. For average sample of each variety were made sawing germination media on liquid medium with distiled water in 2 diferent concentrations of sucrose. The first concentration with sucrose 10%+H_3BO_3 0,01% and the second concentration with sucrose 15%+ 0.01% H_3BO_3. To verify the results both their safety and each experiment were made 3 identical repetitions complete sowing and paralel germination, as shown above. For the risk limitation of contamination and to the prevent environmental deterioration of germination, all utensils, filter paper and culture medium were sterilized in advance. The glass blades germination media in small Petri dishes/plates were maintained at 17ºC to 20ºC in a humid atmospheric so as not to evaporate the liquid medium. It was thus avoides the culture medium concentration by the water evaporation and it was maintained the constant concentration of boric acid (H_3BO_3) and sucrose. The wet atmosphere was maintained by lining the inside of the Petri dishes with paper filter moistened with sterile distilled water (Andrew and Paraschivoiu, 2003). After seeding the first laboratory tests were made after a test period of 5 hours and verification at 24 hours . For microscopic examination were used the optic microscope type IOR ML4 -M. This was

done by transmitted light and ocular phase contrast 10x and objectives 10x, 20x, 40x. The germination was expressed percentage (G%) by the reference of the germinated pollen to the total pollen to grains germinated existing and counted in the field. As in a current practice it was found to be germinated the grains which had pollen tube length equal to twice the pollen diameter at least. The results obtained from the 3 repetitions / experiment there were expressed as percentage (%) based on the appropriate arithmetic mean.

RESULTS AND DISCUSSIONS

The germinative capacity (G%) of the apple tree pollen was made evident on sucrose medium 10% in comparision with 15% sucrose medium. It was found that the Generous variety had 60 %, germinative capacity, double than percentage to Pioneer variety as 30% only. However, it is obvious that in general, the germination (G%) of 30 % is considered as acceptable in terms of production. In the specialized literature it is admitted that germination is representative of the fertility degree of flowers and of fruits binding respectively (Ivaşcu, 2001). The pollen tubes that have germinated in the culture media, have varied lengths of approx. 100µ - 200µ. It was observed that pollen tube length (LTP) is dependent on both internal factors (variety) and external factors (in vitro germination conditions). The most well developed (longer) pollen tubes were identified as follows (Table 1, Fig. 1, Fig. 2):

(a) depending on the variety - Generator variety (LTP 270µ max.) compared with Pioneer variety (LTP 150µ max.);

(b) depending on the concentration of sucrose in the germinative medium- the LTP max 15% sucrose are 150µ and 270µ compared with sucrose 10% and 130µ and 200µ respectively.

The pollen tube length of Generous variety faithfully respond to environmental changes and thus the pollen tubes are longer and therefore no uniform trend. The pollen tubes are not always appropriate for pistil pentrating *(in vivo* The apple germination has some "features/particularities" in terms of development and pollen tube aspect , namely:

- **Type “a” peculiarity (features) -** was found in Generous and Pioner apple variety that pollen tubes can sometimes have forked end.

In this case the two branches ending the pollen tube may or may not equal.

In general, the branching pollen tube has a normal growth in the first 1-2 hours, then there is a branch which is usually about equal to the other portion of the free pollen tube and so the final look is the bifurcation /forking. Both varieties have this "peculiarity" in the apical pollen tube and further testing found the same deviation of the tube morphology and other apple varieties as well (Prima and Florina).

The presence/manifestation of the two types of "peculiarit " in pollen tube development and design, has observed in other apple varieties, which suggests

that it is an "anomaly germination" mainly dependent on species and it is not only an own characteristic peculiarity of the variety.

This kind of germination was observed in three replicates at a rate of 1 to 5% (Fig. 3 and Fig. 4).

Table 1

The action of medium composition on (LTP µ) pollen tube development of Romanian apple Pionier and Generos varieties

Apple varieties	LTP(µ) Medium with 10% sucrose + 0,01% H_3BO_3	LTP(µ) Medium with 15% sucrose + 0,01%H_3BO_3
Pionier	130	150
Generos	200	270

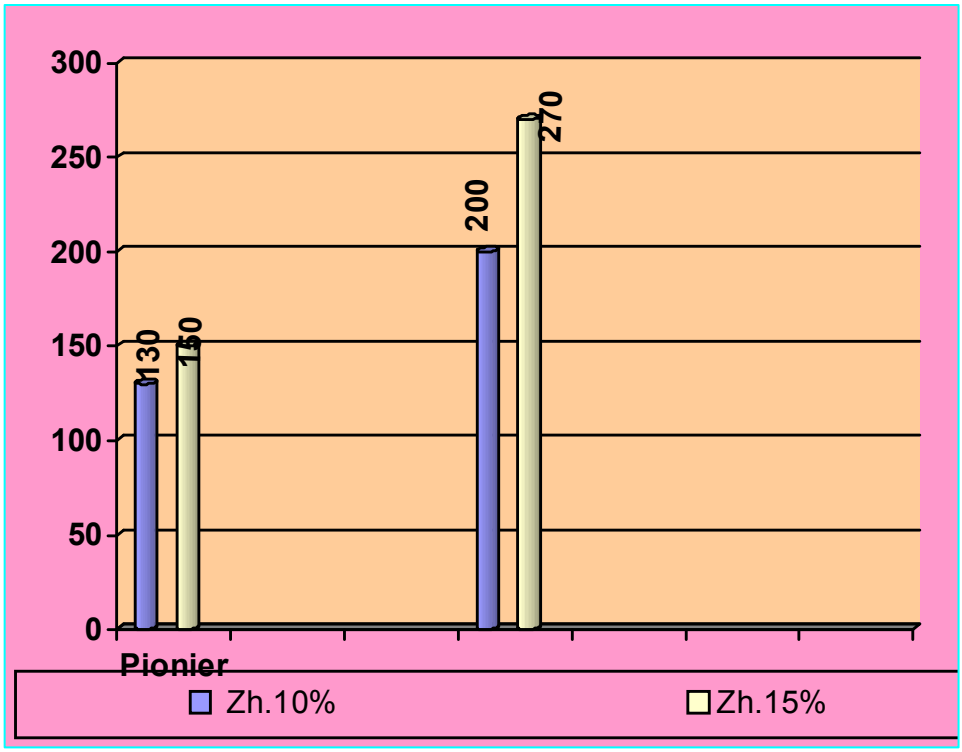

Fig.1 - Dynamic development of the pollen tube (LTP µ) depending on the composition medium (environment) Romanian apple Pionier and Generous varieties.

Fig. 2 - Developing Pollen Tube on Pionier variety medium with 10% sucrose.

Fig. 3 - The type "a" peculiarity- pollen tube with an apical forking in early stage.

Fig .4 - The type "a" peculiarity- pollen tube with apical forking in advanced stage.

•**Type "b" peculiarity**- the second feature consist on the spiraling of the free end (extremity), usually, all in the apical zone. These deviation are more rare than branches (forkings); sometimes, the spiraling is reduced to only one or two spirals or even simple folds of the pollen tube. The fold lines are marked because the two bented portions form right angles or sharp. This type of specific

malformation of pollen tube development was observed in both varieties of apple (Fig. 5) .

Fig. 5 - Type “b” peculiarity with spiraling pollen tube .

CONCLUSIONS

1. The fact that the two types of " features " that have appeared in small percentage of 1% to 5% , in pollen tube development and appearance of both varieties and other varieties that was supplementary tasted sugest us that there is an “anomaly of germination”, especially, dependent on variety species and not only a characteristic of the variety own.

2. The champion of germination in both in vitro envionments was the apple variety Generos with G%= 60% to 15% sucrose medium, double values than the Pionier variety.

3. Usually in the fruit tree practice, they consider pollen germination capacity of about 20 % provides binding fruit with a normal fruit production .

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