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RESEARCHES ON THE MICROSPOREGENESES AND POLLEN TUBE DEVELOPMENT OF SOME CHERRY VARIETIES IN EXPERIMENTAL CONDITIONS

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Abstract

Biological characteristics of pollen formation and development are dependent on weather conditions in the winter and early spring thermal stabilization. In the last years, the weather disturbances manifested by late frosts, affected, mainly, physiological processes in mature pollen, which occurred by the reducing pollen germination capacity both as a percentage and also the development in terms of development in length (LPT) of the pollen tube. It were used specific methods Carnoy fixation for high lighting the microsporogenesis stages and the germination on liquid medium in order to selection optimal variants for the maximum potentialities of the pollen biological value. The work has been done on Romanian varieties of sweet cherry with different periods of ripening: Boambe de Cotnari, Severin, Daria, from Research & Development Station for Pomiculture Baneasa orchards. We observed a normal evolution at the anthers an the trades level of microspores, consisting a good premise for the germinative manifestation capacity of the pollen. In vitro conditions of the developmentdynamics of the pollinic tube varied from 18% at 45% as dominant values. In conclusionwe was considered germinated pollen all the granulates that had pollinic tubes length approximately equal with double the diameter pollen.

Key words: *cherry, length pollen tube, microsporogenesis.*

INTRODUCTION

Microsporogenesis is determined by genetic factors and is dependent or conditioned during his deployment, by sudden thermal fluctuations in winter wich often produce profound disturbances.

Microsporogenesis begins with reduction division (R!) and marks the passage the transition from deep winter rest to optional rest (Bordeianu et al.,1961; Tarnavschi, 1963).

Transition to the tetrad stage, then uninucleate microspores and pollen stage transition to binucleat pollen, depends on

Pollen maturation phase corresponds to the biological threshold (+6.5°C) for swelling buds (Ivaşcu, 2002).

Gonzales et al. (2001), in its study on microsporogenesis, said on the other species economic value, there is no strict relationship between the type and configuration microsporogenesis fourfold orientation is strongly influenced by tree especially during meiosis and there is also no direct correlation between the type and diaphragm microsporogenesis mature pollen grains.

Hedhly et al. (2004), said the pollen germination is the stage preceding the polenic tube growing in stil. Each of these two stages (germination and pollen tube growth is stimulated (driven) by ambient thermal conditions such as: moderate temperature that stimulates the stigma secretion, increases the adherence of the pollen and promotes the germination and the slightly higher temperature accelerate the pollen tube growth and simulates the process of fertilization (Hedhly, 2004).

Concerning nutrition media for the pollen germination consulted in national literature on different species of fruit trees, these contains 1,5% agar-agar (Cociu, Oprea, 1989; Butac et al., 2006; Blidariu et al., 2008), but also with some exceptions such as liquid medium (Iordache et al., 2010).

The object of this paper/research, is the analysis of the pollen through microscopically methods, microsporogenesis determination, degree of maturation pollen by the evolution of sporoderma, the final size from young

microspore to mature pollen, determination/analysis of pollen viability and germination capacity, and the relationship between variations of media and dynamic pollen tube germination, of three Romanian varieties of cherry.
These three varieties of cherry are available in our plantation of fruit trees and have not been investigated (studied) and characterized from this point of view.

MATERIALS AND METHODS

In 2011 there were evaluated microscopically three Romanian cherry cultivars with medium and late ripening period: Severin, Daria, and Boambe de Cotnari. The age of the trees alternates between 7 and 9 years and belong to the Baneasa SCDP collection. The samples consisted of flowering buds and open flowers that were harvested as follows: flowering buds were collected in February-March and in April the flowers were collected in the first day of anthezis and then the flowering buds. Flowering period lasted approximately 7 days.
The flowering shoots were first fixed in Carnoy solution for several hours then preserved in ethanol 70ºC (Andrei et al., 2003).
Open flowers and the buds being opened (balloon stage) were analyzed immediately after harvest (not being necessary or appropriate their setting and preserve).
By dividing into sections (severing) the flowering buds that are being in progress (stage) before the swelling buds (pre-swelling) and swelling of the bud stage (March-April), we obtained the necessary data in the process of observing the early stages of the microsporogenesis (tetrad with microspores, both very young and young microspores, the gradual appearance of the specific elements of sporoderma, the apertures forming, etc.)
The microscopical examination af samples in March has made on permanent preparations in glycerin gelatin, using optical microscopy IOR type ML4-M. It was used objectives 10x, 20x for camera and 40x for microscopic examination.
For better observation (examination) of the morphological elements (features) mentioned, the preparations were stained with Carmin Acetic Acid (ACA) or Methylene Blue vital dye alcoholic solution (Andrei et al., 1972).
They highlighted easier the differences between the microspores with normal maturation of the immatures, were identified viable microspores by the non viables.
At the pollen viability were estimated (V%) viability and germination capacity (G%) for each variety separately. They used anthers extracted from several flowers forming a homogeneons sample that represents faithfully the biological potential of the pollen at that time. Viability (V%) was expressed as a percentage in comparison of the viable pollen with all pollen grains of microscopic fields examined.
To assess (estimate) the capacity of germination (G%), anthers were placed in each small bottle as indicator (watch glass) and few drops of distilled water for hydration for pollen release.
These unessential process and comparable with natural hydration of pollen on the stigma secretion (Xie B. et al., 2010).
The pollen contained in each watch glass was an sample mean for the cultivar examined. From the sample mean of each variety have been sowings of germination media two different concentration of sucrose (15% and 20%).
The culture media that are used to the assess of the pollen germination are liquid media (distilled water) containing and 0,01% boric acid (H_3BO_3).
For each concentration of sucrose were seeded three versions (v_1, v_2, v_3) with which (by means of them) was tasted the action of some flower parts on (upon) germination.
For the safety results were made in all three repetitions (parallel sowing) as follows:

- v_1 – the drop of liquid medium has been seeded only with pollen;
- v_2 – the drop of liquid medium has been seeded with pollen together with pestle;
- v_3 - the drop of liquid medium has been seeded with pollen accompanied by empty anthers.

Pestle was introduced to try a simulation of the conditions *in vivo* referring to the stimulating effects that gineceu induced on the germination release (on the stigma) and then on the development of the pollen tube. Pestle but

could have a negative effect of environmental contamination with saprophytic germs (yeast, molds) and may be itself an undesiderable nutritional support for the development of these germs.
Therefore, similarly I kept 3-5 anthers emptied in the drops (v_3), to pursue these possible negative processes.
After sowing the first laboratory tests were done after an interval of 5 hours.
Microscopic examination of samples taken in April, was done by transmitted light and the phase contrast with objectives 10x, 20x, 40x.
To maintain unaltered microscopic preparations, both microspors extracted from the flowering buds (February-March) and the mature pollen extracted from open flowers or buds in the process of flowering (April) used to assess the viability and germinative capacity, were included after examination, in permanent preparations in glycerin gelatin (Andrei et al., 2003).

RESULTS AND DISCUSSIONS

Concerning microsporogenesis and the development of young microspores, the flowering cherry buds taken in February it was revealed the normal development of anthers in appearance and size during organogenesis in the three varieties studied.
The flowering cherry buds of 8 March – were pointed out (highlighted) tetrades of microspores that characterizes pre-swollen bud stage (before flowering buds swelling). The appearance being normally not observed distartions of the microspores in tetrad or disproportionate development between microspores. It was also observed the uniformity and normality of the cellular content between tetrad components.
The microscopic field were observed and pollen mother cells (PMC), due to non synchronizing of microsporogenesis process (Figure1, Figure 2).

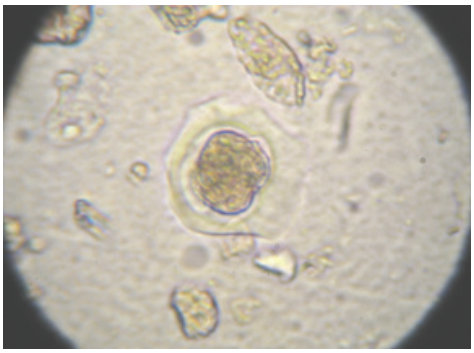

Figure 1. Pollen mother cells (PMC), Ob.20x, Oc.10x

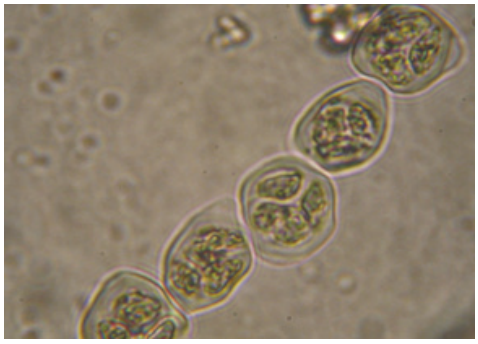

Figure 2. Tetrade chain of microspores in cherry variety Boambe Cotnari, Ob.20x, Oc.10x

On March 18 began to appear very young microspores that still coexist with tetrades and the flowering cherry buds of 21 March it was observed that anthers, microspores very young contains, recently released from the tetrad. Exin contour was devoid of visible ornaments and was examined with objectives 10x,20x,40x.
Contour was approximately circular. Uniform appearance, almost spherical, is explained by the lack of sporoderma stratification.
The apertures not appear obvious just because exin is still thin and devoid (without) ornamentations. In some granules, aperturs are marked by obvious folds.
The flowering cherry buds of 25 March noted tjat microspores are large rand have a maximum size of approx. 23,5 μ. Change in sizes and uneven appearance of microspores are due to non synchronizing of maturation stage of microspores at different anthers of some bud.
There are also differences from one variety to another on the stage of maturation of

microspores at certain date. Microspores are significantly different from those of 21 March as they start to differentiate at sporodermas level weaks ornamentations and we observe the apertures shape. However it also notes the cytoplasmic granulation at the granules with still thin sporoderma .

Concerning mature pollen: as the flowering cherry buds of 6 April, sporoderma has a specific appearance for mature pollen, we proceeded of ots application to test the viability. Were revealed the microspores appearance with a normal development for approx. 70% in deep red and yellow microspores containing reduced or absent cell and pollen with methyl blue staining vital dyestuff alcoholic solution, to highlight the presence of oil droplets that always accompany young pollen maturation (Figure 3 and Figure 4).

Viability and germinative capacity of the three Romanian varieties of cherry have maximum value for version 2 (v_2) with 20% sucrose. Boambe de Cotnari cultivar had viability (V%) 80% and maximum germination (G%) of 48%. Severin variety had viability (V%) 75% and maximum germination (G%) of 25% and Daria variety had viability (V%) 60% and the maximum germination (G%) of 18%.

As currently practiced were considered as being germinated the grains that has pollen tube lenght at least equal twice the diameter of pollen.

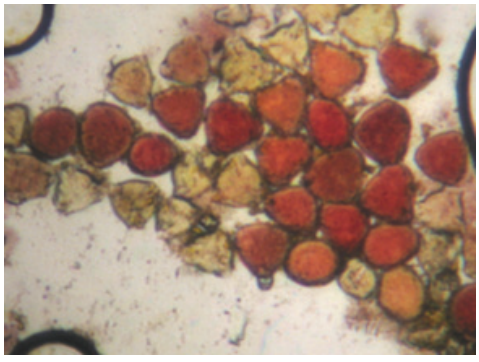

Figure 3. Red color, intense reaction to viable mature pollen during the test for viability, in cherry Severin variety, Ob.10x, Oc.10x.

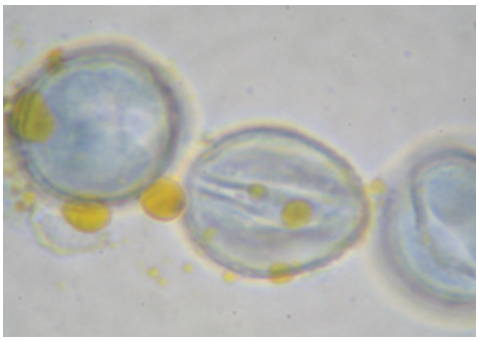

Figure 4. Staining Pollen with methylene blue vital dyestuff alcoholic solution, to highlight the presence of oil droplets that always accompany young pollen maturation in Daria variety, Ob.20x, Oc.10x

The results of 3 repetitions for each experiment, were expressed as a percentage based on the corresponding arithmetic mean (Figure 5).

The pollen tube length (PTL) / variety correlated with average sucrose % in variants v_1, v_2, v_3 are thus: pollen tube germinated at cherry varieties in the Romanian culture media, varies in length from approx. 30µ to about 400µ. (Figure 6).

To determine if a relationship exists between pollen tubes development and the medium the pollen germinated it was formed the graphic which were placed at intervals in order of length (µ) all „PTL max" and the corresponding experiments (Figure 7).

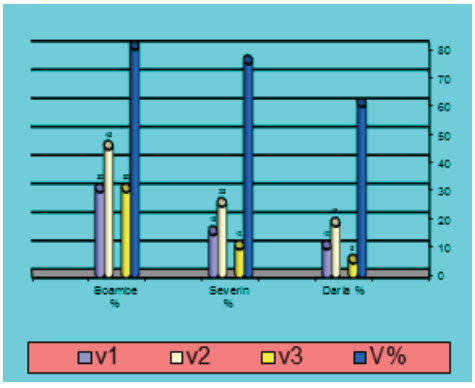

Figure 5. Dynamic pollen (V% and G%) for 3 varieties of cherry in variants (v1,v2.v3) with 20% sucrose.

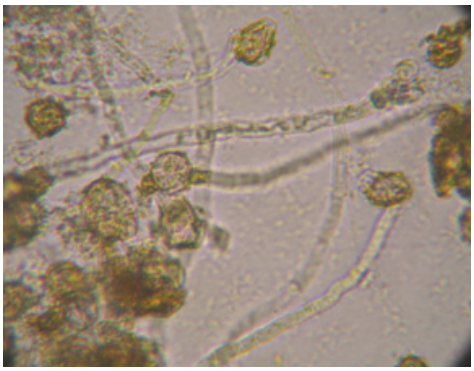

Figure 6. Mature pollen during germination, in cherry Boambe de Cotnari variety , Ob.10x, Oc.10x

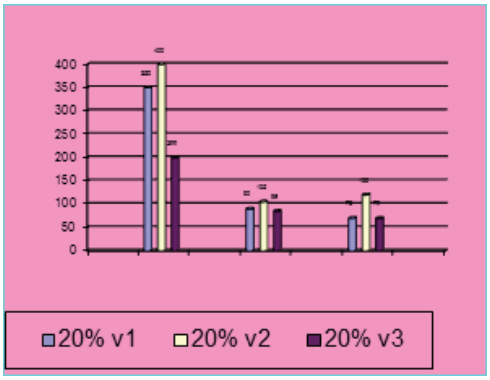

Figure 7. Dynamic pollen tube length (PTL μ) for 3 varieties of cherry in variants (v1,v2.v3) with 20% sucrose

CONCLUSIONS

As a result of these experiments conducted in 2011, in which has been evaluated the biological value of the pollen at 3 varieties of Romanian cherry, we conclude the following:

The pollen maturation went in normal physiological conditions, undisturbed in accordance with the evolution of relatively mild weather winter 2011.

Tetrades and microspores had a normal aspect for the development phasem.

Mature pollen showed viability between 60% and 80% and germination 18% - 45%.

The best germination has been on liquid medium with sucrose 20% and (H_3BO_3) 0,01%.

Boambe de Cotnari cultivar is in the top, both in viability (80%) and in germination (48%) and variety Daria is in the last place with 60% viability and 18% maximum germination.

The best germination of all varieties was obtained in variant (v2/medium + pollen + pestle) with 20% sucrose. Thus confirming the stimulant role of pestle in triggering of germination and pollen tube growth.

The poor germination was v_3 with 20% sucrose and v_3 with 15% sucrose in all varieties. Version 3 (v_3/medium + anthers) usually has a minimum value due to the negative influence of environmental anther tissue on germination.

For version (v_1) 20% sucrose, germination was equivalent to (v_1) 15% sucrose and and had moderate values. Version 1 (v_1/medium + pollen) can be considered indicative value for potential germination of pollen specific granules in the absence of pistle influence.

PTL max. was recorded in variety Boambe de Cotnari (400 μ) also in the version (v2) on an average of 20% sucrose.

The 3 Romanian varieties cherry have brought forth the specific potential of each.

It confirms good germination (18-45%) and the corresponding binding undisturbed weather conditions during flowering and microsporogenesis.

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