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RESEARCHES ON POLLEN VITALITY IN SOME APRICOT HYBRIDS

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Abstract

Pollen vitality as a biological feature is an important factor in the reproduction on amfimictic way and has major implications for the quantity and quality of fruit. In 2012, the negative effects upon the flowering buds that are produced in winter by climatic factors manifested by sudden thermal fluctuations with persistent frost and hard frost, without intermediate temperatures from a period to another have led to strong hurting of buds of fruit. Physiological disturbances due to the low temperatures have had a prolonged effect with repercussions in the microspores maturation, regarding the pollen viability and germinative capacity in some apricot tree. The object of the work is to evaluate the performance in germination %R(G/V) of the pollen, relationship expressed through germinal capacity and of mature pollen viability released from anthers that was affected by frost. There were evaluated microscopically the pollen vitality at apricot tree, hybrids: HB.82.62B.IV. and Nicusor cultivar with a different maturation period from the existing collection of SCDP Baneasa. As a result of the effectuated experiment there were obtained following values: the two biological indicators of mature pollen from apricot, the germinal capacity (G%), viability (V%) and their relation expressed by the performance in germination %R(R/V), on both sucrose environments (15% and 20%),have had values between 11%-33% for germinal capacity (G%), 25%-50% for viability (V%)and for efficiency in germination %R(G/V)between 44%-77% . In conclusion, leaders were the 826.62 hybrid and Nicusor cultivar.

Key words: *germination capacity, pollen tube, viability.*

INTRODUCTION

The assessment of the fertility degree through pollen vitality and "fruit bounded, fruit set" quantity in natural conditions for fruit trees holdings, always involves, the effect of environmental conditions, too.

In vitro experiments of the pollen viability and germination, is practiced method for the fertility estimation of pollen of fruit growing species, (cultivar or hybrid), in forecasting crop, and for to check resistance, tolerance and adaptability pollen (respectively cultivar), to the fluctuations and weather risks (very cold winter frosts return spring frosts, etc.,).

In vitro experiments are a good test for forecasting fertility rates in plantation flowers (Hedhly et al., 2004).

Concentrations of sucrose used by some researchers for the pollen germination testing in vitro environments, were different, (Cociu and Oprea, 1989; Butac et al., 2006; Pădureanu, 2007; Blidariu et al., 2008; Iordache et al., 2010).

Boric acid (H_3BO_3) was added to the medium *in vitro,* at various concentrations (Butac et al., 2006; Iordache et al., 2010).

Both germination and pollen tubes development, are stimulated and depended by ambient thermal conditions.

Temperature during germination, has a determinative effect on pollen tube kinetics and dynamics (both in medium as in style), stimulating then the fertilization process (Hedhly et al., 2004), in comparative study of two cultivars of *Prunus avium* L., Spain and Canada.

The temperatures that for the pollen germination used were were between 18°C, and 25°C (Butac et al., 2006; Pădureanu, 2007; Blidariu et al., 2008).

The favorable temperature of pollen tube development for fruit trees, is between 10°C and 20°C, (Hedhly et al., 2004).

The temperatures below 10°C and above 20°C determine the reduced germination rate and pollen tube development (Hedhly et al., 2004).

The objective of this paper, is to evaluate the potential fertility pollen, in laboratory experimental conditions.

These apricot hybrids experienced in this research, are existing in ours fruit tree plantation and have not been examined / tested and characterized and this point of view. the pollen germination (*in vitro*), in liquid medium (deprived of agar-agar), because we considered it may be relatively comparable, with intercellular fluid composition of stylar tissue, that pollen tube develops, and allow better dispersion of pollen and thus favors in liquid medium, the development of pollen tubes.

MATERIALS AND METHODS

Nicusor cultivar. Age of the trees is 8, with medium and late ripening period. In April (on the first day of anthesis in 04.04.2012), there were taken from open flowers and flowering buds for determing the viability and the capacity of pollen germination.

To asses the viability and to represent as real possible the biological potential of the pollen at that time, to each sample and analysis partly, were used fresh anthers that were extracted from the stem filaments of the flowers or current flowering buds.

directly on microscope slide glass, there were applied (according to the Method Andrei and Paraschivoiu, 2003), directly on the fresh and mature pollen, e few drops reagent 2,3,5-Trifeniltetrazol Clorid, for coloring reaction (Andrei and Paraschivoiu, 2003).

The pollen viability was evaluated under a microscope in transmitted light, by examining of the morphological and physiological characteristics of the pollen cell.

The method is recommending the counting the colored grains only as a result of the deep red reaction (Andrei and Paraschivoiu, 2003).

The viability was expressed in per cent (V%) confronted by the total grains counted in the field by reporting of the viable pollen to total grains of microscopic fields examined.

For creating the media culture *in vitro* to determine / assess germination capacity (G%) of the mature pollen, was hydrated (previously) the pollen, released from anthers fresh , on several slides glass.

This essential process is comparable with natural hydration of pollen on the stigma secretion (Xie B. et al., 2010).

Then separately, were made sowing on germination media in 2 different and separated concentration of sucrose (15% and 20%) in which added 0,01% boric acid (H_3BO_3).

We believe that, the pollen germination advantage on the liquid medium directly on slide glass, allows at better microscopic examination through transparency and the transmited light unlike the germination on solid medium in Petri dishes, which cannot be examined only in direct light at o "power enlargement" of max. 200x for stereo magnifier.

Also the pollen germination testing, was done in 3 different germination the tasting in 3 germination variants (v1,v2,v3) by introducing of some floral parts (ginaeceum / pistil and the remaining emptied anthers), in germination medium to emphasize their influence / action on pollen germination (Iordache et.al., 2010).

So each microscopic slide was a test variant (V1,V2,V3) thus as follows:

- Variant V1- liquid drops was seeded only with pollen
- Variant V2 – drop of liquid was seeded with pollen together pistil, for a simulation of the conditions *in vivo*, relating to the stimulating effects that gynaeceum induces on starting of germination (on stigma) and then on pollinic tube development.
- Variant V3 – liquid drop was seeded with pollen together remaining emptied anthers for to have in view these possible negative processes.

Slides with media were kept at on average temperatures 17°C to 20°C in wet atmosphere so that the liquid medium doesn't evaporate and thus it is maintained constant concentration in boric acid and sucrose.

To reduce the risk of environmental contamination and to avoid the deterioration of germination medium, all the tools with which they were working, including filter paper and cultural medium were sterilized previously (Andrei and Paraschivoiu, 2003)..

After sowing, the first laboratory tests were made after a period of 5 hours of testing and 24 hours.

As currently is practiced they were considered germinated grains that had pollen tube length at least twice the pollen diameter.
To point out the pollen tube and nuclei were applied specific colorings on germination media with dilluate solution of Methyl Blue. (Andrei and Rădulescu, 1972).
The germinative capacity of the viable pollen was expressed as a percentage (G%) based on the corresponding arithmetic media.
Then the values of germination (G%) were reported, the viability (V%) corresponding to the hybrid / cultivar to obtain of the efficiency in the germination of pollen % R(G/V).
After the microscopical preparations, the germinated pollen was included in glycerin jelly to maintain the microscopical preparation unaltered (Andrei and Paraschivoiu, 2003).
For microscopic examination was used optical microscope IOR type ML-4M.
To point out the viability and the pollen germination, examination, assessments and photographs were made in transmitted light, polarized light, and objectives 10x, 20x, 40x and C.F. (phase contrast) with 10x ocular.
It was used ocular micrometer to measure polinic dimensions.

RESULTS AND DISCUSSIONS

Regarding pollen viability evaluation (V%): viability ratio (%) is the first condition in pollen germination and gives us information about the probability of fertilization of the pistil.
After the application of the specific enzymatic method for the determining viability (V%) of the pollen, the intense red color reaction has occurred at the following hybrids of apricot: Hybrid 82.6.62.B.IV. had maximum of approx. 50% and at the apricot Hybrid 82.28.62.B.IV. has registered a low value of approx 25%, having as leaders / having in view the viability), the Hybrid 82.6.62.B.IV. and Nicusor cultivar (Table 1, Figure 1).
The viable mature pollen grains at apricot, had the following dimensions: from 48,3µ - 50,1µ in the equatorial optical section (image obtained in polar view) and from 44,0 µ - 46,3 µ in optical meridian section (image obtained in equatorial view).

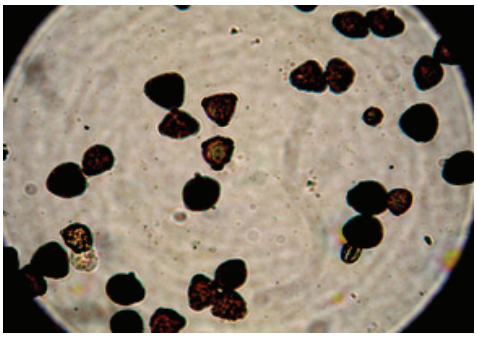

Figure 1. Viability at Nicusor apricot cultivar, 05.Apil.2012

It was found that in terms of size and morphological aspect and the apertures development, the germinal grains, so, with the normal physiological development have dimensions close to those typical of the same species (Tarnavschi et al., 1981-1984).
Regarding evaluation of germination (G%) by the action of pollen composition averages on 15% and 20% sucrose: the germinative capacity (G%) of each hybrid and cultivar individually has expressed by two maxim values corresponding germination on the two medium with 15% and 20% sucrose, to variant (v2).
The highest values of germination capacity (G%) were at the Hybrid 82.6.62.B.IV. and Nicusor cultivar, (table 1, figure 2, figure 3): 28% and followed by 33% on sucrose medium 20% followed by 33% and 37% on sucrose medium 15%.
It is a remarkable fact that from the point of practical view, in general the germination (G%) in 30% is considered satisfactory, because the specialized literature recognizes that this germination is representative for the fertility degree of the flowers, respectively the binding of future fruit (Ivascu, 2001).
Therefore, germination value (G%) presented in table and graph, are the maximum values recorded for each hybrid, to certain concentration of the medium with sucrose included in the experiment.

Table 1. Relationship between germination G% viability and germination yield % R(G/V) at the apricot cultivars and hybrids evaluated in 2012

Hybrid/cultivar	MATURE POLLEN				
	Germination max.(G%) of total pollen		Germination max.(G%) of total pollen	Germination max.(G%) of total pollen	
	Zh 20%		*Zh 20%*	*Zh 20%*	*Zh 20%*
HB 82.28.62.B.IV	11	15	25	44	60
HB 82.4.41.B.IV	17	19	30	57	63
HB 82.6.62.B.IV	28	33	50	56	66
NICUŞOR	33	37	48	68	77
(*)The viability is determined by coloring with 2,3,5 Trifeniltetrazolclorid (**)The germination productivity (% RG/V) is calculated by the ratio of germination/viability (%)					

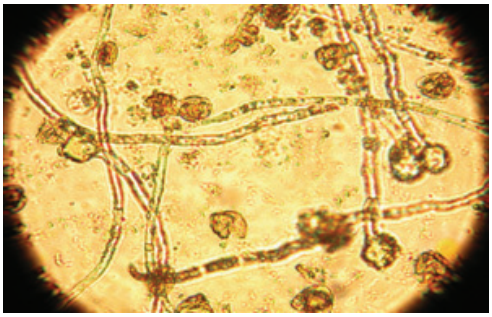

Figure 2. Apricot hybrid 82.662.BIV. on medium, with 115% sucrose (04.April.2012)

Regarding the evaluation of pollen germination (G%) by the action of experimented floral parts in 3 variants (v1,v2,v3), on pollen germination on the mediums with 15% and 20% sucrose: the maximum values obtained depending on the variants are representative for pollen germination from each of the hybrids studied.

The highest germination values are observed at in second variant (V2/medium+pollen+pistille) and it is confirmed the incentive role of the pistil (at both mediums with 15% and 20% sucrose), except apricot hybrid 82.28.62.B.IV., where V1>V2 on both environments.

Generally the size relation between the three variants (v1,v2,v3) is the following:

- Variant (V2/medium+pollen+pistil) has maximum value because the incentive role of the pistil in the germination and the pollen tube growth.
- Variant (V3/medium+anthers), has a minimum value as a result of a possible negative influences on germination that the anthers tissue has.
- Variant (V1/medium+pollen), has an average value (in the absence of favorable influence of the pistil, and the absence of the negative influence of the anthers) and can be considered the indicative value for the specific germination potential of pollen grain.

The percentage germination (G%) appears correlated with viability (V%) respecting the natural relationship between them.

For each hybrid and cultivar as well, the germination value were assigned to the viability values (V%), separately for germination medium with sucrose 15% and separately for the germination medium with 20% sucrose.

In this way was given by the productivity in germination % R(G/V), that expressed the degree of correlation between the two biological characteristics of the pollen (the power of germination and pollen viability) and the germination relation/viability expresses as germinable, viable the pollens are, because not everything is viable has the power/ its ability to germinate.

The highest potential in germination %R(G/V) is 77%, the leader being Nicusor cultivar of apricot Hybrid 82.6.62.B.IV. on average of 15% sucrose and the lowest %R(G/V) is 44% for Hybrid 82.28.62.B.IV. on average 20% sucrose .

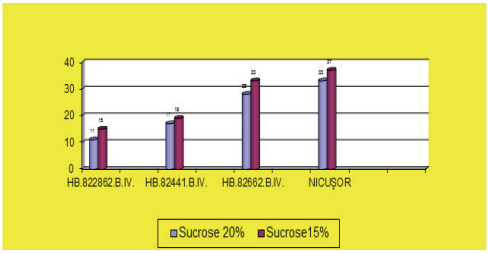

Figure 3. Action composition average 15% and 20% sucrose on pollen germination of apricot cultivars and hybrids evaluated (2012)

CONCLUSIONS

From experiments in 2012 in which was evaluated the viability (V%), the germination capacity (G%) and the efficiency in germination %R(G/V), we have drawn the following conclusions:

The flowering of the apricot this year started in the first days of April.

The investigated apricot cultivars and hybrids have yielded in 2012 accordingly to the specific and individual potential but with negative influences due to the microsporogenesis that was damaged in part and poor flowering in small proportion.

Regarding the two biological indicators of the pollen, the viability V% and germination G% and their relationship expressed by efficiency in germination %R(G/V), on both sucrose medium (15% and 20%), were obtained the following values:

The highest germination yield %R(G/V), it has Nicusor apricot cultivar on 15% sucrose medium that presented 77% R(G/V) in terms of only 48% viability (V%) and 37% germination (G%).

The best germination percentage regarding the action in media composition were recorded on liquid medium with 15% confronted with 20% sucrose medium.

The highest values of germination and also of experienced floral representative parts in 3 variants (v1,v2,v3), has consistency two variant (v2/medium+pollen+pistil) under the stimulant influence of pestle on both environments.

Therefore the variant V2 we consider more concluding, because of the interaction between pollen and pistil for binding fruits prognoses than V1.

In vitro conditions, the germination (G%) of the pollen expresses by the pollen fertility potential and this relationship germination viability expresses as germinable and vigorous are viable pollens and the germination degree %R(G/V) express the correlation degree between these two biological characteristics of the pollen.

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