POLLEN MORPHOLOGY, POLLEN'S GERMINATION CAPACITY AND VIABILITY OF FOUR BLACKCURRANT CULTIVARS (*RIBES NIGRUM* L.)

MORFOLOGIA, CAPACITATEA DE GERMINARE ŞI VIABILITATEA POLENULUI A PATRU SOIURI DE COACĂZ NEGRU (*RIBES NIGRUM* L.)

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Abstract: *The research was conducted in 2010 and aimed at establishing the biological value of pollen of four varieties of black currant (three romanian varietes and one foreign variete): Deea, Abanos,Ronix, Tsema. Pollen viability was found between 64.9% and 86.39 %, and ability to germinate with values between 42.07% and 81.7%. These studies revealed a close link beetween pollen size and pollen germination capacity. Also to quantify results in terms of free pollination and self-pollination. Percentage of flowers setting fruit in free-pollination of self-pollination condition was 15-22% lower than in free pollination.*

Key words: blackcurrant (*Ribes nigrum* L.), pollen grain, viability, germination, self pollination.

Rezumat: *Cercetările s-au efectuat în 2010 şi au urmărit stabilirea valorii biologice a polenului a patru soiuri de coacăz negru (3 româneşti şi unul străin): Deea, Abanos, Ronix şi Tsema. S-a constatat o viabilitate a polenului cuprinsă între 64.9% şi 86,39%, şi o capacitate de germinare cu valori între 42.07% şi 81,7%. Aceste cercetări au relevat şi o strânsă legătură între dimensiunea grăuncioarelor de polen şi capacitatea de germinare. De asemenea s-a determinat coeficientul de autofertilitate şi fertilitate naturală. Procentul de fructe legate în condiţii de autopolenizare a fost cu 15-22% mai mic decât în cazul polenizării libere.*

Cuvinte cheie: coacăz negru (*Ribes nigrum* L.), polen, viabilitate, germinare, autopolenizare.

INTRODUCTION

Ribes nigrum L. is the species of currant with greatest economic importance of the genus *Ribes*. The quality of yield depends on several agrotechnical and pedoclimatic factors, but also the pollination process. Literature indicate major differences between processes of pollination and processes of fertilization. Pollination is conditioned on the kind of pollen vector, while fertilization depends on the number of pollen grains reaching the stigma (Kołtowski et al., 1999).

The most varieties of black currant are self-fertility, but to be emerged differences between the varieties what may have genetic causes, such as higher position of stigma versus the anthers, especially the flowers on top of racemic (Kołtowski et al., 1999, cited by Denisow B, 2003). Viability of pollen grains and weather conditions are other aspects to be considered in assessing the fertility of varieties. One consenquence of low relative humidity of air is the dehydrating pollen. This is

prevented by species-specific adaptation mechanisms or even variety. Drought during pollen development in anthers can strongly to affect viability of pollen (Bots and Mariani, 2005 cited by Davarynejad et al 2008). Determination of viability and germination capacity of pollen are important in selection of suitable polliniser, because not all pollen can reach to the eggs (Davarynejad et al 1993).

The ojectives of this study were to investigate the viability and germination capacity of grains pollen *in vitro,* knowledge of the biological value of pollen for selection of suitable pollinisier. By determining in free-pollination and self-pollination of the percentage of flowers setting fruit we studied the characterization of varieties in terms of fertility index. In the same investigation was performed also the study of the pollen grain morphology with a focus on the pollen grain size.

MATERIAL AND METHOD

The research was conducted in Iassy, 47°10' - 47°15' N 27°30'E, Romania, in spring 2010 using the biological material collected from four self-fruitful blackcurrant varieties (three Roumanian varietes and one foreign variety): Deea, Abanos, Ronix şi Tsema.

Pollen germination capacity was determined *in vitro* by on solid nutrient medium (17g sucrose, 1.5 g agar, 0.01 g of boric acid in 100 ml of distilled water) at 20-22^{0}C and a relative humidity of 70 - 90%. Germination energy was calculated as a percentage for each set of 10 measurements / variety (Cociu V. and Oprea Şt., 1989) after 24 hours.

Pollen viability was evaluated microscopically after staining with carmine-acetic. The stained pollens in red were considered as viable in these tests. The sterile pollen remains colorless or stained in pink (Cociu V. and Oprea St., 1989, and Botu Botu , 1997).

Self-fertility was expressed as percentage (number of flowers setting fruit x 100/ number of isolated flower) (Cociu V. and Oprea St., 1989, Botu and Botu, 1997).

To establish ***the natural fertility*** were the flowers at least 200 of each variety (50 in the four cardinal points), these branches were tagged and fruits were the results (Cociu V. and Oprea St., 1989).

Experince was conducted by randomized block method in four replications with six plants per plot.

For each variety was measured the transversal diameter (td) and the longitudinal diameter (ld) for 50 pollen grains on glycerin jelly slides each.

All observations, measurements and photos of blackcurrant pollen were made on the light Motic Microscope under x10, x40, x100 magnification.

For statistical interpretation of results we used the coefficient of variation (s%) which was accepted arbritar following values: 0-10% - lower coefficient of variation, 10-20% - mean coefficient of variation, 20-30% - great coefficient of variation.

RESULTS AND DISCUSSIONS

Phenological stages and weather conditions:

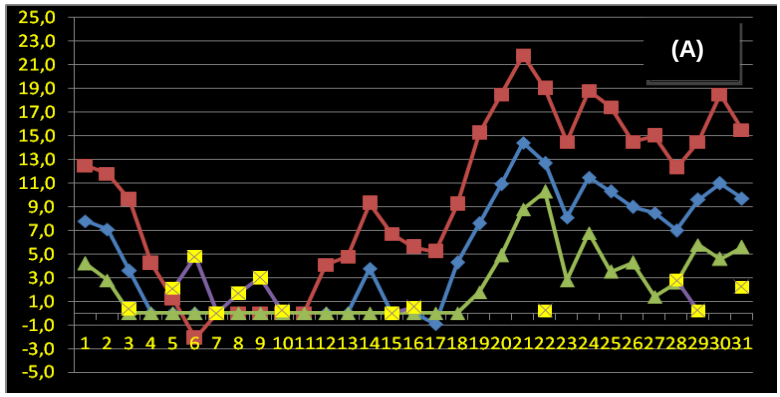
The bud burst appeared during the third week of March (table 1). In March 2010 the average air temperature was 4.3^{0}C, average absolute minimum air temperature -7.5^{0}C and average absolute maximum 21.,8^{0}C. The baloon stage of blackcurrant started in first of April. In April 2010 the average temperature was 11,9^{0}C. The blackcurrant varieties have different flowering times: Deea was the early blooming varieties in 3-April, while the Tsema flowers in 15-April which were the late blooming varieties. In during of flowering period the air temperatures dropped

below 17^{0}C, which determinated to extend this phenological stage. The flowering period took approximately 10-18 days. Beginning of the third decade of April (April 23) was marked by the temperatures below 0^{0}C. In this period the petals of Deea var. Fell and and the first fruit set. Rainfall recorded in March and April not quantitatively significant, the total being 18,1mm and 28mm (figure 1).

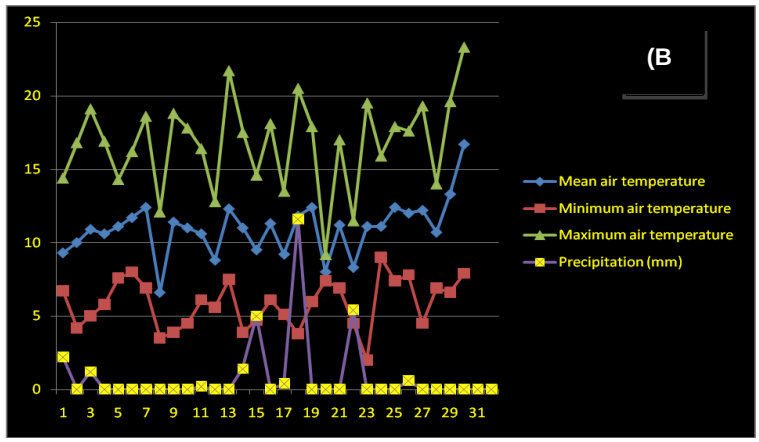
Table 1

Phenological stages of black currant under climatic conditions of Iassy, Romania (2010)

Varieties	Bud burst	Balloon stage	Full bloom	First fruit set
Deea	15 Mar.	17 Mar.	3 Apr.	20 Apr.
Abanos	18 Mar.	19 Mar.	5 Apr.	24 Apr.
Ronix	21	22 Mar.	7 Apr.	24 Apr.
Tsema	28	1 Apr.	15 Apr.	1 May



1. A



1 B.

Fig. 1 (A and B). Weather conditions during the bud burst period and flowering period of blackcurrant: March (A) and April (B) 2010

Biological value of pollen grains of black currant varieties:

The mean percentage germination ability and the percentage viability of pollen grains was differed significantly between varieties (table 2).

Table 2

Biological value of of pollen grains of black currant varieties

Variety	Germination ability of polen (%)	Viability of polen (%)	Percentage of flowers setting fruit in *free pollination* (%)	Percentage of flowers setting fruit *self pollination* (%)
Deea	81.7	86.3	47.0	32
Abanos	69.1	86.2	65.0	46.2
Ronix	42.07	64.9	39	26.4
Tsema	81,6	86,4	70,7	48,6
s %	17,97	19,45	17,55	16,64

The percentage of viable pollen to black currant varieties studied ranged between 64.9% and 86.4%, Ronix variety registering the lowest values. Researchs conducted over several years by Szklanowska et. al. (1997) shows that blackcurrant pollen viability does not depend on the cultivar. Our results can be explained by the existence of adverse climatic conditions during microsporogenesis.

After examining of data on germination capacity is observed that the values obtained in the varieties Tsema (figure 2) and Deea are close to the percentage of viable pollen (more than 80%). Lowest percentage germination (42.07%) was obtained from Ronix var.

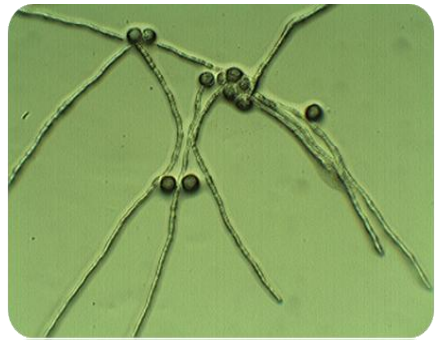


Fig. 2. Germination pollen of Tsema var.(MOTIC MICROSCOPES ob x 40)

The coefficient of variation (s%) of pollen grains viability for blackcurrant varieties analyzed was 19.45% and the coefficient of variation of pollen germination capacity was 17.97%. These results allows us to appreciate that there is a medium variability for these indicators of biological value of pollen.

The percentage of fruit setting varied depending on the pollination method and the variety of currant and (table 2). This index of the biological value of pollen had a coefficient of variation (s%), below 20%, indicating an medium variability. Highest values were obtained for the free pollination: Abanos var. 65%. and Tsema var. 70.7%, in agreement with the point of view of many authors

(Grădinariu G., 2002, Denisow B., 2002) about the importance of insects and their positive influence in the process of pollination in black currant.

At the same varieties, the self-ferility coefficient was 46.2% to Abanos var. and 48.6% to Tsema var., which shows that self-pollination does not guarantee a better fruit set even varieties with a high percentage of self-fertility. The index of natural fertility for Deea var. has had 47%, close self-fertility index (32%), which may be due to unfavorable conditions at the end of flowering or susceptibility of style.

Pollen size: After were measured of the lenght the pollen grain of blackcurrant varieties it was found that the longitudinal diameter (LD) and the transversal diameter (TD) are not equal, bud the raport LD/TD is close to 1 (table 4). The pollen grain of blackcurrant is circular and nonangular (figure 3) in accordance with the authors Wrońska-Pilarek (1998).

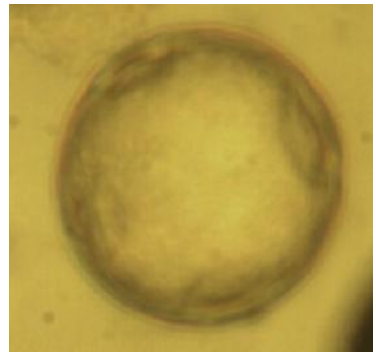


Fig. 3. Pollen grain of Tsema var. (MOTIC MICROSCOPES ob x 100)

The average lenght of the transversal diameter (TD) of the pollen grains varied in the range of 17.1 µm to 19.2 µm and the average lenght of the longitudinal diameter (LD) was between 18.1 µm to 20.3 µm.

Table 4

Size of pollen grains of blackcurrant varietes (µm)

Variety		Deea	Abanos	Ronix	Tsema
average	LD	18,7	18,9	18,1	20,3
	TD	17,9	17,9	17,1	19,2
	LD/TD	1,04	1,06	1,06	1,06
min	LD	14,6	16,1	13.7	16,6
	TD	13,7	15,6	13	18,0
max	LD	21,4	21,9	27,6	22,6
	TD	21,5	21,7	26,5	21,6

**Longitudinal diameter (LD)* ***Transversal diameter (TD)*

CONCLUSIONS

This researchs showed that there were differences between varieties of black currant regarding the biological value of pollen probably caused by unfavorable weather conditions (especially temperature) during of microsporogenesis and pollen tubes growth. The percentage of fruit set depended on the method of pollination and the currant variety.

The highest values of the self-fertility coeficient was: 48.6% on Tsema var., provided that the same varity of the natural fertility coeficient was 70.7%. The high values for germination capacity and pollen viability recommend the variety Tsema as best polliniser between varieties analyzed.

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