

**POLLEN GERMINATION OF SOME POMEGRANATE (*PUNICA GRANATUM*L.)  
VARIETIES GROWN IN MONTENEGRO**

Miroslav IZMOVIC<sup>1\*</sup>, Ranko POPOVIC<sup>1</sup>, Biljana LAZOVIC<sup>1</sup>, Mirjana ADAKALIC<sup>1</sup>,  
Ahmed DŽUBUR<sup>2</sup>

<sup>1</sup>Biotechnical Faculty Podgorica, Montenegro

<sup>2</sup>Agromediterranean Faculty Mostar, Bosnia and Herzegovina

\*(Corresponding author: miroslaw@t-com.me)

**Abstract**

The research on pollen germination of three most important pomegranate varieties grown in coastal region of Montenegro and the hinterland is presented in this paper. The trial was carried out during the three consecutive years (2002-2004) on two sweet ('Slatki barski' and 'Šerbetaš'), and one sour ('Dividiš meke kore') pomegranate varieties. Pollen germination was tested on solid 1 % agar medium (Agar Plate method) with two concentrations of sucrose (10 and 15 %). 'Slatki barski' and 'Šerbetaš' varieties showed higher degree of germination at the 15 % sucrose medium (65,96 and 71,27 %) than at the medium with 10 % (42,41 and 39,70 %). The degree of pollen germination was significantly lower in 'Dividiš meke kore', on both sucrose concentrations (16,5 and 22,54 %). Therefore, pollen germination parameter in this variety should be tested before planting in mono-varietal orchards.

**Key words:** pomegranate, *Punica granatum* L., pollen, germination

**Introduction**

Pomegranate (*Punica granatum*L.) is commonly grown as a shrub or small tree that can grow up to 5 m. For successful development requires dry and hot summers, with relatively mild winters, although in the tropics is evergreen (Ozguven & Yilmaz, 2000). It was considered as a symbol of fertility, and often quoted in the Bible and the Koran (Mars, 2000). Origin of pomegranate species is usually connected to the area of former Mesopotamia. Zohary and Hopf (2000), however, mainly due to the richness of diversity of wild pomegranate in the Mediterranean area, the countries such as Albania and Montenegro cited as a possible wider gene center of this species. Most of the authors classify pomegranate into *Punicaceae* family, although in the official databases of gene banks it is classified in the family *Lythraceae*. Recent molecular studies (Stover et al., 2007) confirmed this taxonomic affiliation of pomegranate.

Pomegranate, along with olive and fig trees belongs to the longest cultivated fruit species in this area. There is no precise data on the age of this species in our region, but the area of Bar in seventies of the last century had 90 000 of pomegranate bushes (Plamenac, 1977). The number of bushes decreases in the recent period due to the transformation of agricultural land into urban area. There was no evidence of the annual production of this species in Montenegro. However, the worldwide trend in increasing the number of pomegranate trees is obvious, and it is projected to be soon among the leading ten fruit species (FAO, 2010).

Pomegranate forms two types of flowers: hermaphrodite (complete) which is the only able to bear fruit, and functional male flowers which only contribute to a better pollination. Although it is considered to be a self-pollinated plant, much better fruit set was observed when pollinated with cross-pollination (Josanetal., 1979). The aim of this work was to investigate the germination of pollen grains of the most important pomegranate varieties in the

Montenegrin subtropical zone, with purpose to recognize the yield potential and the possibility of establishing mono-varietal plantations.

### Material and methods

The investigation was conducted on three prevalent pomegranate varieties in Montenegro: ‘Slatki barski’, ‘Dividiš’ and ‘Dividiš meke kore’. Pollen of each of three varieties was collected from three different locations (shown on the map 1): Dobra Voda (DV) and Tomba (MN) in the coastal region, and Balabani near Golubovci (ZP) in Zeta-Bjelopavlici valley. The abbreviations used in the paper for the varieties and the localities are presented in the following table.

Table 1. Abbreviation for pomegranate varieties and sampling localities

Abbrev	Variety - locality	Abbrev	Variety - locality
SBMN	Slatki barski - Tomba	ŠMN	Šerbetaš - Tomba
SBBB	Slatki barski - Dobra Voda	ŠBB	Šerbetaš - Dobra Voda
SBZP	Slatki barski - Balabani	DMK	Dividiš meke kore - Balabani



Map 1. Localities of pomegranate plantations used for pollen sampling

Each of the varieties was presented with 5 trees per locality. All trees were of same age (13 and 14 years) and in the full productive period.

Pollen was collected from the flowers which were kept at room temperature in a bowl with water, up to the moment of anthers rupture. Collected pollen was kept in tubes in desiccators before sowing (Melgarejo et al., 2000). The media for pollen germination contained sucrose at two concentrations, 10 and 15 %, with 1 % agar. Germination was carried out in a hermetically sealed Petri dish at a temperature of 28 °C (dryer Hereaus) in the dark. Observation and reading of germination was done after 12 hours on the light microscope with a magnification 100x and in three visual fields. The degree of germination was calculated as percentage from the ratio of germinated and the total number of pollen grains in the visual field.

Statistical analysis was performed by analysis of variance as a two-factorial experiment: genotype in a given locality (factor A) and years of research (factor B). Comparison of differences of mean was tested by Tukey's test for the significance of 0,05 and 0,01 %.

### Results and discussion

#### Pomegranate pollen germination in 10 % sucrose solution

Pollen germination depends on many internal and external factors. A high percentage of pollen grains germination determined in the laboratory conditions may indicate its ability to be good pollinator (Nini -Todorovi , 1989). Successful pollination and its good fertilizing power directly determine the number of seeds in the fruit, and thus its largeness (Derin & Eti, 2001).

Table 2. –Pollen germination in 10 % of sucrose solution, comparison of differences

Variety (A)	Year (B)			$\bar{X}_A$	HSD <sub>A</sub>
	2002	2003	2004		
S. barski DV	41,77 ab	34,51 b	42,95 ab	39,74 a	HSD <sub>0,05</sub> = 4,6011 HSD <sub>0,01</sub> = 5,5062
S. barski MN	42,46 ab	34,97 b	41,66 ab	39,70 a	
S. barski ZP	39,75 ab	33,93 b	41,34 ab	38,34 a	
Šerbetaš DV	46,33 a	38,39 ab	42,52 ab	42,41 a	
Šerbetaš MN	41,60 ab	35,81 ab	42,95 a	40,12 a	
Dividišmekekore	16,39 c	15,13 c	18,02 c	16,50 b	
	HSD <sub>0,05</sub> = 9,8378		HSD <sub>0,01</sub> = 11,316	F(A)= 0,0000** F(B)= 0,0000** F(AB)= 0,7301 <sup>ns</sup>	
$\bar{X}_B$	38,05 a	32,12 b	38,24 a		
HSD <sub>B</sub>	HSD <sub>0,05</sub> = 2,6598		HSD <sub>0,01</sub> = 3,3496		

Analysis of variance showed that the cultivars statistically significantly differ in the percentage of pollen grains germination in 10 % sucrose solution (table 2). Variation in germination in the years of study has also demonstrated a highly statistically significant difference, while the interaction of these two factors was not significant.

Comparing differences in mean percentage of germinated pollen grains show a statistically significant and significantly lower germination of pollen grains in the year 2003 in all of the tested varieties (32,12 %). Germination in 2002 and 2004 was approximately at the same level (38 %). The lowest pollen germination was in variety 'Dividiš meke kore' (16,5 %), highly significant and significantly lower than in the other tested varieties. Highest germination was in 'Šerbetaš' variety, the average for three years was 41,30 %.

#### Pollen germination in 15 % sucrose solution

Analysis of variance of pollen germination in 15 % sucrose solution showed statistically significant differences between varieties. The year as factor studied also showed statistically significant differences, while the interaction of these factors had no statistical significance.

On the base of the results presented in Table 3, it can be concluded that the lowest pollen germination was in 2003 (53,66 %) in all varieties, and thus highly significant and significantly lower than in the other two years of research.

The highest percentage of germinated pollen grain was registered in 'Šerbetaš' variety from locality Dobra Voda (71,27 %), significantly higher when compared to the varieties 'Slatki barski' and 'Dividiš meke kore' from location Balabani.

Table3 - Pollen germination in 15 % of sucrose solution, comparison of differences

Variety (A)	Year (B)			$\bar{X}_A$	HSD <sub>A</sub>
	2002	2003	2004		
S. barski DV	71,46 ab	58,14 ab	68,27 ab	65,96 ab	HSD <sub>0,05</sub> = 7,316 HSD <sub>0,01</sub> = 8,755
S. barski MN	66,90 ab	59,26 ab	62,53 ab	62,90 ab	
S. barski ZP	62,59 ab	54,47 b	67,50 ab	61,52 b	
Šerbetaš DV	72,67 a	68,12 ab	72,92 a	71,27 a	
Šerbetaš MN	70,90 ab	61,29 ab	68,19 ab	66,79 ab	
Dividišmekekore	27,14 c	20,67 c	19,82 c	22,54 c	
	HSD <sub>0,05</sub> = 15,642		HSD <sub>0,01</sub> = 17,993		F(A)= 0,0000** F(B)= 0,0000** F(AB)= 0,5274 <sup>ns</sup>
$\bar{X}_B$	61,95 a	53,66 b	59,87 a		
HSD <sub>B</sub>	HSD <sub>0,05</sub> = 4,229		HSD <sub>0,01</sub> = 5,326		

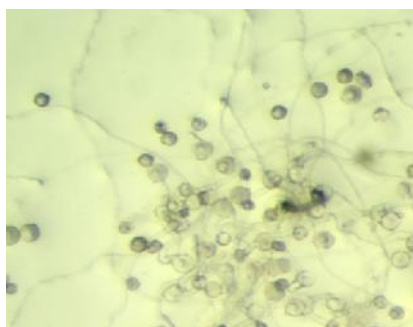


Photo 1 – Pollen germination in 15 % sucrose medium in ‘Šerbetaš’ variety

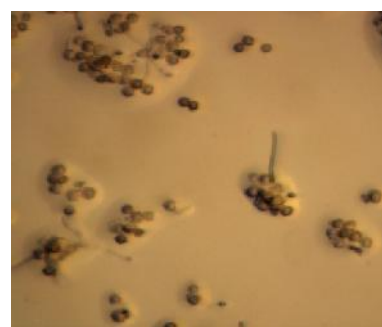


Photo 2 – Pollen germination in 15 % sucrose medium in ‘Dividiš meke kore’ variety



Photo 3 – Pollen germination in 10 % sucrose medium in ‘Šerbetaš’ variety

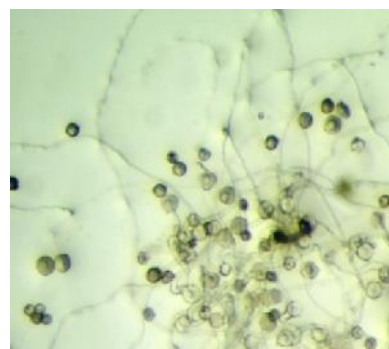


Photo 4 – Pollen germination in 15 % sucrose medium in ‘Slatki barski’ variety

Considering the threshold of significance 95 %, ‘Šerbetaš’ variety from Dobra Voda location had significantly higher percentage of germinated pollen grains in comparison to ‘Slatki barski’ from Tomba site and the other two tested varieties. The lowest germination was detected in ‘Dividiš meke kore’ variety, highly significant and significantly lower when compared to all other varieties (photo 2). The low percentage of pollen grains germination in ‘Dividiš meke kore’ variety is pointing out the possible problems when grown in mono-varietal plantations due to the reduction of fruit set as a consequence of poor fertilization. In three-year study period the lowest germination was in 2003 that may be a consequence of unusually high temperatures during the flowering period. Maximum temperature in May and

June was 30,6 and 35,7 °C in Bar and 32,4 and 38,5 °C in Balabani, which is higher of average temperature for this region.

The difference in the average pollen germination observed in relation to the sucrose concentration was smallest in 'Dividiš meke kore' variety, only 6,04 %. The highest difference in pollen germination related to this factor was recorded in 'Šerbetaš' from Dobra Voda site (28,86 %).

Best germination of pollen grains were registered when the grains were grouped close to each other (Photos 1 and 4), also reported by Pejkić (1998). This phenomenon is considered to be a consequence of greater amounts of secreted enzymatic material and other stimulus, which form "bridges" and connect the pollen grains in the immediate vicinity.

Similar results in examining pollen germination in pomegranate other authors have reported. Džubur (1999) stated that the pollen germination in examined wild pomegranate biotypes ranged from 19,68 % on 10 % sucrose solution (hanging drop), up to 72,98 % in 15 % sucrose solution. Also, the difference in pollen germination among the study years was noted. Wetzstein et al. (2011) while examining pollen germination of pomegranate variety 'Wonderful' in 12 % sucrose solution concluded that the germination depends to a large extent on the temperature conditions. Important is to note that 0,062 % of CaNO<sub>3</sub> and 0,024 % of boric acid was added into the sucrose solution. The highest percentage of pollen germination (74 and 79 %) was registered at 25 and 35 °C, and that there were no significant differences in germination of pollen isolated from anther of hermaphroditic and functionally male flowers. Increase of temperature up to these values increase the pollen germination, while the higher and lower temperatures had negative effects on germination. Pollen grains from both types of flowers had spherical shape and length of ≈ 20µm.

The addition of micro elements, especially B, significantly increased the percentage of pollen germination (Pejkić, 1998). Similar allegations were given by Derin & Eti (2001) while examining the pollen grain germination of 'Hiczas' variety and clone 31 N 06. The authors stated that the production of pollen grains is higher in functionally male flowers, whose function is in better fertilization. Nalawadi et al. (1973) as the best for testing pollen germination suggested the 10 % sucrose medium, while the results of Chitale and Deshpande (1970), showed the highest germination in the medium with 20 % sucrose.

Studying the pollen germination in *Rosa dumalis* and *Rosa villosa* Seazai (2007), by quoting other authors, concluded that pollen germination depends on the environmental conditions as well as on good condition and nutritional status of the plant.

### Conclusion

The highest degree of pollen germination was in 'Šerbetaš' variety from Dobra Voda locality (71.27 %) at 15 % sucrose solution. Pollen germination was high in all the years of research in 'Slatki barski' variety.

'Dividiš meke kore' variety had significantly lower pollen germination in both sucrose solutions and in all three years of research, suggesting the necessity to provide another variety as pollinator to obtain better fertilization and fruit set.

Germination of pollen grains varied considerably, depending on the environmental conditions in some years.

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