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RESEARCH ARTICLE

BIOLOGICAL SPECIFICS OF MALE GAMETOPHYTE IN FEIJOA SELLOWIANA BERG

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ABSTRACT

The research deals with the biological specifics of the development of the male gametophyte in Feijoa sellowiana Berg. 1% agar-agar solution and solutions of sucrose of three different concentrations (20%, 30%, 40%) were used for the investigation. We germinated pollen gains in the Moist Chamber van Tieghem (hanging drop); to paint the pollen grains, the acetocarmine method was applied. Based on the results of the research the low fertility of the Feijoa pollen grains has been defined. 12 hours after having the seeds sowed in all cases only 1-2% of the pollen grains were germinated, whereas 34-47% was germinated after 24 hour period. In this case 40% sucrose solution appeared to be optimal. In case of sucrose solution of 20 and 30% the amount of the germinated pollen grains mounted 13-24% within 24 hours after being sowed. The shape and size of the pollen grains change less in accordance with being depended on the forms of Feijoa. In 98% cases the pollen grains have a triangular form. We also come across with roundish, elliptic and oval pollen grain forms. Their sizes range between 221- 262 mcm^2.

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INTRODUCTION

Feijoa (Feijoa sellowiana berg) belongs to Myrtaceae Family. The homeland of Feijoa is South America (Brazil, North Argentina, Paraguay, Uruguay). Wild Feijoa populates in the forests of South America (as the sub-forest) This is the typical subtropical plant of the dry air. Therefore, it is successfully cultivated in the humid subtropical zone conditions in Georgia. The habitat of Feijoa mainly is comprised of two republics: Georgia and Azerbaijan. Under the conditions in Georgia the major number of the plants ispresented in Samegrelo, Guria and Ajara. In Azerbaijan the majority is presented in Lankaran and Astara (Kedelidze, 2013). A.Feijoa Sellowiana Berg is a diploid species 2n=22 in agreement with the basic chromosome number for the Myrtaceae x=11. The genome size is about 245 Mbp/1C and the DNA content is 0.252 pg/1C what has led to consider A. sellowiana as a'very small'genome species. These values are similar to that reported for P.guajaba (2n=22; 247Mbp/1C; But significantly smaller when compared with Eucalyptus grandis (2n=22; 611 Mpb/1C; A. sellowiana is a predominanty outcrossing species with a mechanism of late-acting self-incompatibility related to post-zygotic rejection. Therefore, no inbred lines are available (Quezada *et al.,* 2014).

Moreover, antioxidant activities of an aqueous extract on oxidative burts of human whole blood phagocytes as well as isolated polymorphonuclear leukocytes have been reported. In addition, Feijoa peel contains components exhibiting MDR-modulation, These biological activities are interesting as emerging drug resistance is present among bacteria, virus and tumor cells. Finally, the tropical and subtropical countries where Feijoa is daily consumed have lower cancer incidence. Some anticancer activities of the full Feijoa extract have been reported, but a complete characterization of its active principles is lacking (Bontempo *et al.,* 2007).

International Institute of plant genetic resources /Bioversity international/ elaborated descriptors for over 80 plants culture based on which the evaluation of the species considers the complex-cytological study, investigation of molecular and biochemical characteristics, biotic and abiotic stresses and

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their resistancy to be the necessary condition (Vashakidze and Maghradze, 2008).

Available resources reveal that at about 70% of flowering plants are hermaphrodite plants and the amount of sterile pollen grains in each of them does not exceed 5-10 %, that provides the basis to consider such grain to be fertile. Sterile pollen is within the norms in 30% of flowering plants out of which defectiveness of pollen of approximately 10% of species is connected to sex structure differentiation and in 3-5% -to further hybridization, polipoidia, cytomplasmic male sterility and various types of influences of the environmental factors (Cacenko, 2010).

It is possible to investigate Feijoa pollen grain viability in vitro culture as well as frozen condition (-18C^0) (Correa *et al.,* 2005). As the resources prove it could be effected by the following various factors: breeding types, internal species hybridization, cytoplasmic sterility and set of anthropogenic factors (Cacenko, 2012).

The male sterility (self-incompatibility) is often named to be the reason of sterility. If the plants are far from each other cross-pollination does not take place and relevantly the fruit set does not take place.

We think that the main reason of sterility or low productivity is the non-homogeneity (diversity) of forms resulted through seed breeding. One of the reliable reserves of increasing the productivity of plantation is to reveal, investigate and embed the self-fertile forms in production (Adamadze, 1985).

Male sterility in Feijoa can be of cytoplasmic nature (cytoplasmic male sterility-cms). Two types of the stated sterility is described in the plants:

1. Total male sterility (plant genotype cit^s xxzz andcit^s xxZZ).
2. Yellow pollen dust contain fine pollen grains, moreover they do not tend to open andpollen is not exposed (plant genotype cit^s Xxzz, cit^s xxZz, cit^s XXzz, cit^s xxZZ).

Visual inspection and investigation of the anthers of Feijoa reveal two different types of anthers:

1. The anthers are much bigger in size and the pollen grains developed in have brighter yellow color.
2. The anthers are much smaller in size and the pollen grains developed in are relatively colorless, the opening and pollen grain exposure is not observed. Therefore, Feijoa is characterized with the second type of sterility.

Normal pollen grain is developed in all plants, under the normal cytoplasm conditions (cit^N), as well as in the following four genotypes with the sterile cytoplasm: XxZz, XXZz, XxZz, XxZZ, XXZZ (Pukhalski, 2007).

In our opinion the main cause of the sterility or low productivity in Feijoa is the non-homogeneity (variety) of forms resulted through seed breeding. One of the reliable reserves for increasing the plantation productivity is to reveal, investigate and embed the self-fertile forms in production.

MATERIALS AND METHODS

The object of the research is Feijoa (Feijoa sellowiana berg),which populates in three ecological zones in the Western Georgia: (Ajara, Guria and Samegrelo). In order to investigate the biology of the distinguished forms of pollen grains in Feijoa we applied 1% agar-agar solution and sucrose solutions of three different concentrations (20%, 30%, 40%). The pollen grains were germinated in moist chamber van Tieghem (hanging drop); We painted them with acetocarmine paint (Pukhalski, 2007). We took records of the amount of the germinated pollen grains in 12 and 24 hours after being sowed.

We picked flowers from 12:00 p.m. after the sun rise and from the plants where 15% of the buds were already opened. In order to investigate the viability of the plant the pollen was sowed on the object glass, with 20%, 30%, and 40%-of nutrient medium. After having the seeds sowed on the object glasses we placed them on Petri Dishes made moist artificially and put them in 30C^0 thermostst. The amount of the germinated seeds was recorded after 12 and 24 hour period, to set it in the permanent status we used to dehydrate and degrease the preparation. To make the preparation permanent we used biomount (Pukhalski, 2007; Cacenko, 2010).

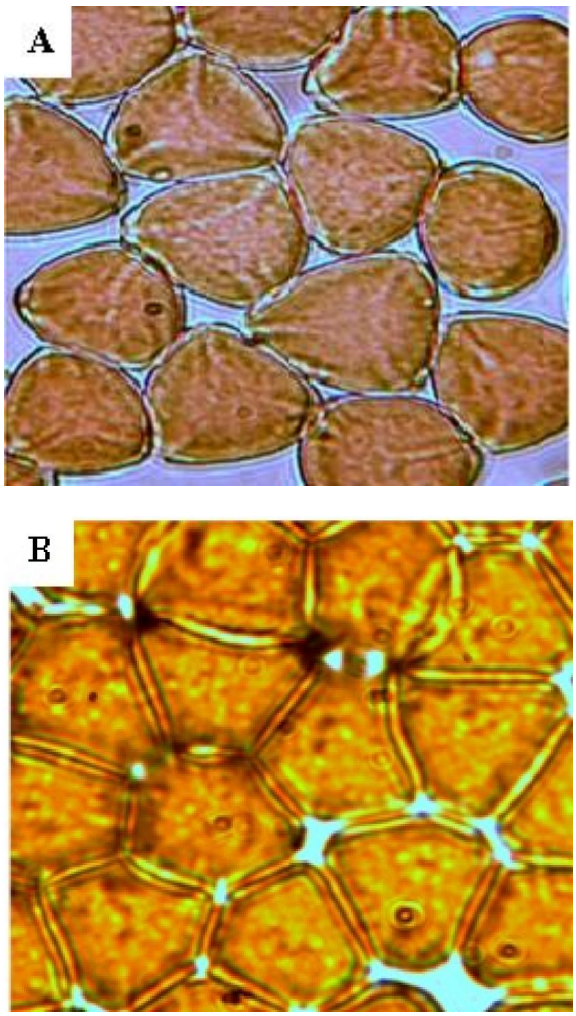

Figure 1. Feijoa Grains Painted with Hematoxylin (A) and Acetocarmine (B)

When applying the paint through acetocarmine method the mature pollen grains were fixed in acetamide ethyl with correlation 3 (ethyl), 1 (glacial acetamide). The fixation lasted for 2 -12 hours. We washed the material after fixation and stored it in 80% ethyl. The material to be examined was shifted to the object glass; We put the drops of acetocarmine, smashed it, made it free from any extra tissues, put the glass cover, warmed it up at 70-80^0 (1 minute) and examined under the microscope (Fig.1. B).

Pollen grains were also painted by applying haematoxylin. We shifted pollen anthers from 80% ethyl to the object glass, put drops of haematoxylin, smashed it, made free from the extra tissues, put the glass cover, warmed it up and examined under the microscope (Fig. 1. A).

To reveal the self-fertile forms we focused on two regions: Ajara and Guria, particularly Supsa and Batumi Botanical garden. In order to carry further study of the examination 300 fruiting plants were singled out. In order to exclude cross-pollination possibility in May of the following year (2009), during the flowering period in May we isolated the branches of the plants, being observed, with 0,06 m^2 size sacks of bandage. 2-3 branches were isolated for each plant. In autumn we recorded the number of developed fruits for the isolated branches and total number of flowering plants.

The experimental data were elaborated through the method of variational statistics using special computer software (Graph pad prisma 6).

The aim of the investigation also was to define germination energy, form and size, fertility and sterility and germinability of pollen grains of various forms of Feijoa species called Choiseana through applying various nutrient medium.

RESULTS AND DISCUSSION

As it is clear from the Figure 2 the forms of the Feijoa pollen grains are various (roundish, ellipse, oval, etc.) 98% of them are triangle. The difference among pollen grains in size is minor (221-mcm^2), however in comparison to the data to be controlled this difference is more essential (Fig. 2). According to the results of the analysis carried out based on the ecological zones any reliable statistics on differences in size and form of the pollen grains have not been recorded. After having applied various nutrient medium it was defined that the germinability of the Feijoa pollen grain is influenced by the sucrose concentration. The data given in picture 3 reveal that the % of germination, in 12 hours after having sowed the Feijoa pollen grains, is low in all three ecological zones and ranges between 1,2% and 2.0%. Besides it is maximum after the application of 40% sucrose.

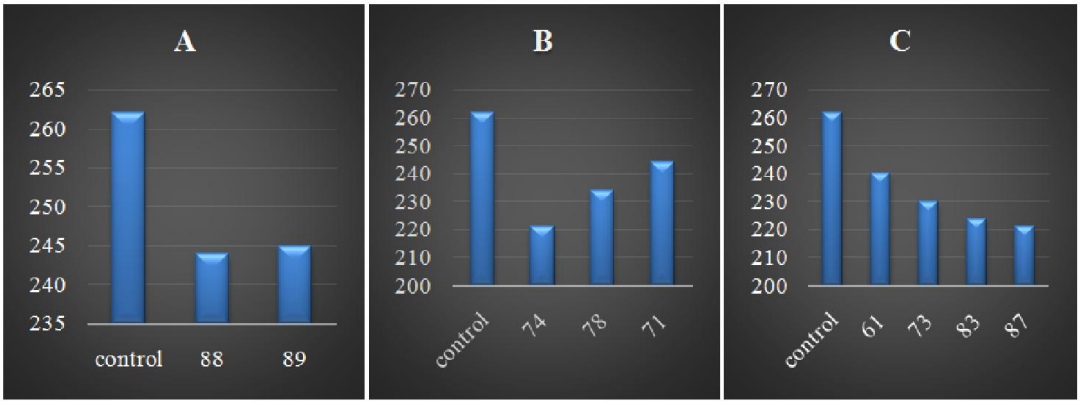

Figure 2. Changeability of Feijoa pollen grain size (mcm^2) within various forms according to the ecological zones: A-Ajara; B-Guria; C-Samegrelo

Table 1. Germinability (%) of Feijoa form pollen grain populated in Ajara, Guria and Samegrelo and the germinability energy when applying different concentrations of sucrose

Regions	Names of forms №	In 12 hours after beging sowed Concentration 20 %	30 %	40 %	In 24 hours after beging sowed Concentration 20 %	30 %	40 %
Samegrelo	61	1.10±0.00 p<0.0001	1.40±0.02 P<0.1481	2.00±0.02 P<0.2630	13.60±0.03 p<0.0001	16.00±0.2 p<0.0001	34.60±0.13 p<0.0001
	73	1.20±0.01 p<0.0001	1.50±0.02 p<0.0046	1.90±0.00 p<0.0287	15.50±0.01 p<0.0003	18.30±0.05 p<0.0001	36.40±0.46 p<0.0001
	83	1.11±0.01 p<0.0001	1.30±0.03 P<0.0185	1.80±0.03 P<0.0047	14.60±0.11 p<0.0001	16.70±0.22 p<0.0001	34.30±0.10 p<0.0001
Guria	87	1.20±0.02 p<0.0001	1.10±0.02 p<0.0001	1.50±0.01 p<0.0001	13.60±0.04 p<0.0001	15.50±0.15 p<0.0001	34.80±0.07 p<0.0001
	74	1.30±0.01 p<0039	1.60±0.03 p<0007	1.80±0.01 p<0.0001	16.20±0.03 p<0.0001	17.40±0.14 p<0.0001	35.7±1.30 p<0.0001
	78	1.20±0.03 p<0.0005	1.2±0.01 p<0.0001	2.01±0.06 P<0.8799	15.80±0.05 p<0.0001	18.10±0.27 p<0.0001	35.8±1.35 p<0.0001
	71	1.20±0.01 p<0.0001	1.30±0.03 p<0.0262	1.70±0.04 p<0.0011	16.10±0.03 p<0.0001	19.40±0.15 p<0.0001	36.70±0.10 p<0.0001
Ajara	88	1.10±0.01 p<0.0001	1.5±0.03 P<0.0185	2.0±0.07 P< 0.2080	17.60±0.10 p<0.0001	20.10±0.03 p<0.0001	36.70±2.00 p<0.0009
	89	1.30±0.03 P<0.0222	1.40±0.03 P < 0.2054	1.90±0.01 P< 0.0378	18.04±0.19 p<0.0001	21.70±0.07 p<0.0001	37.20±1.11 p<0.0001
	Control	1.40±0.01	1.40±0.01	2.00±0.05	20.10±0.01	24.30±0.11	47.10±0.21

In 24 hours after being sowed the % of germination is significantly high in case of all three concentrations of sucrose, however it is the highest (35-47%) in case of 40% sucrose concentration (Table 1).

At the end of the vegetation period we carried inspection of the plant being under the observation (the status of the fruit development on the isolated branches). As the results demonstrated, out of 300 plants the fruits from the isolated branches developed only for five plants. Consequently they represent self-fertile plants. We carried repeated experiment. In this case the complete isolation of all five plants was made with the bandage. The result was the similar to those of the previous years. The results obtained proved that the amount of self-fertile forms, in the plantations cultivated through Feijoa seeds, equals to 1, 7%.

Based on the above mentioned, the investigation results make us conclude that the male incompatibility of Feijoa is caused by cytoplasmic male sterility. The percent and energy of the pollen grain germination is relevantly low. Despite the male self-incompatibility, in the plantations cultivated with seed plants, the amount of self-sterile as well as self-fertile and of those later does not exceed 1.7%. Considering the provided data vegetative breeding of forms and their embedment in the production is possible.

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