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In vitro pollen germination of feijoa (*Acca sellowiana* (Berg) Burret)

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ABSTRACT - *The objective of this work was to establish a suitable culture medium and conditions to be used for* in vitro *germination tests of feijoa pollen. It also had the aim to test the possibility of storage of this pollen in a freezer (-18 °C). The pollen was collected from flowers immediately after anthesis. The test media were the standard (10% sugar, plus 1% agar dissolved in distilled water), and the addition of two H_3BO_3 (0.65 mM and 1.3 mM) to this medium. Incubation temperatures of 25 and 30 ºC were tested. High average percentage of* in vitro *germination (79.7%) was obtained after three hours of incubation at 25 °C. Highest germination percentages (85.6, 52.3 and 9.3% after storage for zero, 90 and 150 days respectively) were achieved with the addition of 1.3 mM of H_3BO_3 to the standard medium. Feijoa pollen lost viability after 90 and 150 days in freezer storage, reducing germination percentage from 79.7 to 45.9% and 6.1% respectively.*

Key words: Myrtaceae, culture medium, boron, pollen storage.

INTRODUCTION

The feijoa (*Acca sellowiana* (Berg) Burret), also known as pineapple guava, is a myrtaceae fruit plant native of South America, more specifically of the Southern Brazilian States (Ducroquet et al. 2000). This species has great potential for commercial use, mainly because of the fruit characteristics.

Years ago, Embrapa Temperate Climate Research Center, in Pelotas, RS, Brazil, began a research program aiming at future breeding programs of native fruit species, for their inclusion in commercial plantings.

Information of the pollen viability, which will be used in hybridizations, is an important item in a breeding program. However, there is no available information about ideal conditions for testing pollen viability through *in vitro* tests, for the majority of wild fruit species. Only a few studies with some species of *Psidium* (including the Brazilian strawberry guava) were found in literature review (Hirano and Nakasone 1969, Raseira and Raseira 1996).

In order to estimate pollen viability, four methods can be used: 1) *in vitro* germination tests; 2) use of dye, stain method; 3) *in vivo* germination by observing pollen tube growth on the stigma and in the pistil and; 4) seed formation after normal pollination of a selected female parent (Galletta 1983).

The staining method is the fastest and easiest one, and is generally used. However, it generally overestimates

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the pollen viability, because often unviable pollen grains can be stained due to their enzymes or starch content or even other substances. The two latter methods however, are very time and work demanding in order to have results (Galletta 1983).

The *in vitro* method is the most convenient and is able to estimate the reserve's substances and membrane conditions as well as the reserve conversion for pollen germination (Marcellán and Camadro 1996). Besides, this is the most used method in breeding programs.

The *in vitro* pollen germination is influenced by several factors, such as the species, the culture medium, the temperature and time of incubation, the flower development stage when collected, in addition to storage conditions (Stanley and Linskens 1974). There are differences among species and among cultivars within species, in reference to the germination medium and required conditions (Rosell et al. 1999).

The most common medium utilized for *in vitro* pollen germination is constituted by sugar and boric acid (Miranda and Clement 1990), to which other nutrients can be added (Galletta 1983). The sugar addition has the objective of providing osmotic equilibrium between the pollen and germination medium, as well as being an energy source to aid the pollen development process (Stanley and Linskens 1974). The boron in the culture medium stimulates the pollen tube growth, however the results vary among species. Boron interacts with sugar, giving origin to a sugar-borate complex, which can act more rapidly on the cell membranes (Pfahler 1967).

The objective of the present work was to determine the culture medium and conditions to be used in viability tests of feijoa pollen, as well as to observe the possibilities of storing in the freezer.

MATERIAL AND METHODS

Flowers were collected at balloon stage and right after anthesis, from several plants of a seedling population collected in Southern Brazil, and now part of Germoplasm Bank of native fruits of Embrapa Temperate Climate Research Center, Pelotas, RS, Brazil, in order to have a representative sample of the *Acca sellowiana* population.

Anthers were detached and placed in paper trays, under environment temperature (20 to 25 °C), for three days, until dehiscence. The ones collected from balloon stage did not dehisce and consequently did not release pollen.

Pollen collected from flowers just after anthesis was divided in two parts: one used for germination test while the other portion was stored in a desiccator with silica and placed in freezer at -18 to -15 °C and low humidity.

The following media for in vitro pollen germination were tested:
10% sugar + 1% agar (standard culture medium);
10% sugar + 1% agar + 0.65mM borax (H_3BO_3);
10% sugar + 1% agar + 1.3mM borax (H_3BO_3);

The medium constituent substances were dissolved in distilled water and warmed in microwave in order to completely dissolve the agar. The media were distributed on slides previously prepared for this objective (common slides used in routine microscopy adapted with two PVC rings of 21 mm diameter and 3 mm height), instead of slide chambers. Each PVC ring represented one experimental plot.

Pollen was sprayed over the culture medium and the slides were then placed in simulated humid chamber (Petri dish with humid paper towel). These Petri dishes were taken to a temperature controlled BOD type oven (25 or 30 °C). Percentage pollen germination counting was done three hours later, considering germinated all the pollen grains which tubes were equal or larger than grain size. One hundred pollen grains were counted in each plot.

New *in vitro* tests were performed, in the same way already described, 90 and 150 days after storage, in order to verify if the storage conditions were suitable for maintaining the pollen viability of this specie.

The statistical design was completely randomized, in a factorial arrangement, with four replications. A variance analysis was performed and the means were compared by Duncan test (P=0.05). The data were transformed using *arc sin* $\left(\sqrt{x/100}\right)$. All statistical analysis was realized with the Sanest software (Zonta and Machado 1984).

RESULTS AND DISCUSSION

The highest percentage of feijoa pollen germination was obtained by adding 1.3mM H_3Bo_3 to the standard medium and using 25 °C as the incubation temperature (Table 1). There was not a significant interaction between the studied factors.

The percentage of pollen germination was high (74.7%), as well as the pollen tubes length (over 20 times the pollen grain diameter) after three hours incubation, indicating that this period is enough to evaluate feijoa pollen viability through *in vitro* test, since a pollen grain

with a tube equal or larger than its diameter is considered germinated.

The required incubation period varies according to the species, or even among cultivars within a species. A longer period was required by strawberry guava (*Psidium cattleyanum* Sabine), another myrtaceae native fruit tree of Southern Brazil. Raseira and Raseira (1996) found that an incubation period between four to six hours at 25 °C temperature was needed. However, Teoatia et al. (1970) estimated a period of 13 hours of incubation in order to have pollen tube emergence. Also, Hirano and Nakasone (1969) evaluated the germination of several *Psidium* species, 12 hours after inoculation on the culture medium.

Average percentage of *in vitro* feijoa pollen germination was lower at 30 °C, showing that high temperatures are not suitable for viability tests of this species.

Incubation temperatures around 25 °C are cited in the literature for a large number of species. Thompson and Batjer (1950) used 24 °C for testing the pollen of plum, peach, apricot and cherry. For Black walnut (*Juglans nigra* L.), Hall and Farmer (1971) used 27 °C. Twenty five degrees Celsius is also considered ideal for avocado (Loupassaki et al. 1997). The results of the present paper showed that 25 °C is also adequate for *in vitro* germination tests of feijoa pollen.

The feijoa pollen partially lost its viability after being stored for 90 days in a freezer and had a great loss after 150 days. The average germination was generally higher on the medium with 1.3mM of H_3BO_3 (Table 2). The interaction among the different factors was not significant.

The Brazilian strawberry guava pollen (*P. cattleyanum*) germinated better *in vitro* on a medium with boron at 0.65 mM H_3BO_3 (Raseira and Raseira 1996). Hirano and Nakasone (1969), however, used 1.63mM of H_3BO_3 for the same species.

Varying results to boron addition to culture medium were observed in black walnut (*J. nigra* L.) (Hall and Farmer 1971). Two clones of this species had higher germination percentage when 1.63 mM of H_3BO_3 was added to the medium while for another clone, the results were independent of the boron presence or absence.

The European nut (*J. regia* L.) pollen had better germination on culture medium containing 0.16mM of H_3BO_3 (Luza and Polito 1985). According to the same authors, small amounts of boron added to the culture medium improve germination, pollen tube growth and reduce the probability of their disruption.

In the present experiment, the feijoa pollen lost almost completely its viability after 150 days of being stored. Pollen storage is important in a breeding program since it has to be viable until the time to be used in hybridizations. The

Table 1. Percentage of *in vitro* pollen germination of feijoa (*A. sellowiana*), collected from flowers right after anthesis, on different culture media and temperatures, after three hours incubation

Culture medium*	Incubation temperature*		Germination means for culture
	25 °C	30 °C	medium[1] %
10g sugar	72.4	66.8	69.6 B
10g sugar + 0.65mM H_3BO_3	80.3	67.4	74.1 B
10g sugar + 1.3mM H_3BO_3	85.6	74.3	80.3 A
Germination means for temperature of incubation (%)[1]	79.7 a	69.6 b	-
Coefficient of variation (%)	6.38		

[1]Means followed by different small letters on the lines and capital on the columns differ by Duncan test (P=0.05)
* Non significant interaction among factors

Table 2. Percentage of *in vitro* pollen germination of feijoa, collected from flowers just after anthesis, on different culture media and storage periods under freezer conditions (-18 °C), evaluated after three hours incubation at 25 °C

Culture medium*	Incubation temperature*			Germination means for culture
	00	90	150	medium[1] %
10g sugar	72.4	45.2	2.6	35.6 C
10g sugar + 0.65mM H_3BO_3	80.3	40.2	7.5	40.7 B
10g sugar + 1.3mM H_3BO_3	85.6	52.3	9.3	48.1 A
Germination means for temperature of incubation (%)[1]	79.7 a	45.9 b	6.1 c	-
Coefficient of variation (%)	8.27			

[1]Means followed by different small letters on the lines and capital on the columns differ by Duncan test (P=0.05)
* Non significant interaction among factors

maintenance of the pollen germination capability depends on inner characteristics as well as storage conditions.

According to the literature, there are differences among species in reference to the maintenance of pollen viability. The viability of plum pollen, evaluated by *in vitro* tests, was maintained for three years after storage at -20 °C. However, if stored at -1 °C, the viability was drastically reduced within 11 months (Lee et al. 1981).

Completely different results were observed in European nuts (Luza and Polito 1985). The pollen of this species greatly reduced its viability after three months of storage at -20 °C. Based on the short pollen tubes obtained, *in vitro*, these authors considered that viability loss is associated with reduction of pollen vigor.

Viability of Brazilian strawberry guava pollen (*P. cattleyanum*) was considerably reduced within 21 days of storage (Raseira and Raseira 1996), under the same conditions tested here for feijoa.

More tests with feijoa pollen should be performed, especially the ones related with storage conditions, since it is desirable to have maintenance of viability for longer periods than the ones obtained here.

In the present work, there was no anther dehiscence when flowers were collected at balloon stage, therefore no pollen liberation occurred. Special procedures should be taken when pollen has to be collected from opened flowers. One of then is to bag the flowers at balloon stage, using wax paper bags, in order to avoid pollen contamination with others. Another way is to cut twigs with flowers at balloon stage and bring then indoors, maintaining twig base in water until the flowers open and pollen can be collected.

CONCLUSIONS

1. The viability of feijoa (*Acca sellowiana*) pollen can be evaluated by the *in vitro* method using a culture medium with 10% sugar, 01% agar, dissolved in distilled water, and incubated for three hours at 25 °C.
2. Addition of 1.3 mM of H_3BO_3 improved *in vitro* germination percentage.
3. It is possible to maintain a reasonable viability of feijoa pollen up to 90 days of storage in freezer at -18 to -15°C. New storage conditions and pollen collection procedures must be studied.

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Germinação *in vitro* de pólen de feijoa (A*cca sellowiana* (Berg) Burret)

RESUMO - *O objetivo do presente trabalho foi verificar qual o meio de cultura e condições que deveriam ser utilizadas em testes de germinação* in vitro *do pólen de feijoa, bem como verificar a possibilidade de seu armazenamento em congelador (-18°C). O pólen foi coletado de flores logo após a antese. Os meios de cultura testados foram o padrão (10% de açúcar e 1% de ágar em água destilada) e este acrescido de duas concentrações de H_3BO_3 (0,65 mM e 1,3mM). Foram testadas as temperaturas de incubação de 25 e 30 ºC. Três horas de incubação, a 25 °C, proporcionou alta percentagem média de germinação* in vitro *(79,7%). A adição de 1,3 mM de H_3BO_3 ao meio de cultura resultou nas maiores percentagens de germinação (85,6, 52,3 e 9,3%, ao zero, 90 e 150 dias de armazenamento, respectivamente). O pólen de feijoa apresentou perda de viabilidade após 90 e 150 dias de armazenamento em congelador, reduzindo a percentagem média de germinação de 79,7 para 45,9% e 6,1%, respectivamente.*

Palavras-chave: Myrtaceae, meio de cultura, boro, armazenamento de pólen.

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