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In vitro viability of genipap pollen grains in different culture media

Abstract – The objective of this work was to evaluate the viability of genipap (*Genipa americana*) pollen grain at room temperature in different culture media. The experimental design was completely randomized in a 3x11 factorial arrangement (culture media x incubation times) with four replicates. The number of germinated pollen grains was analyzed at 24-hour intervals up to 288 hours after incubation at room temperature. The culture medium with 100 mg L^{-1} H_3BO_3, 80 g L^{-1} sucrose, and 1.0 g L^{-1} agar results in a higher to intermediate germination percentage, being the most suitable for studies on the in vitro viability of genipap pollen grains.

Index terms: *Genipa americana*, in vitro pollen germination, plant breeding, pollen tube.

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Viabilidade in vitro de grãos de pólen de jenipapeiro em diferentes meios de cultura

Resumo – O objetivo deste trabalho foi avaliar a viabilidade in vitro do grão de pólen de jenipapeiro (*Genipa americana*) à temperatura ambiente, em diferentes meios de cultura. O delineamento experimental foi inteiramente casualizado em arranjo fatorial 3x11 (meios de cultura x tempos de incubação), com quatro repetições. O número de grãos de pólen germinados foi analisado em intervalos de 24 horas até 288 horas após incubação em temperatura ambiente. O meio com 100 mg L^{-1} de H_3BO_3, 80 g L^{-1} de sacarose e 1,0 g L^{-1} de ágar resulta em alta à média percentagem de germinação in vitro, sendo o mais adequado para estudos da viabilidade in vitro de grãos de pólen de jenipapeiro.

Termos para indexação: *Genipa americana*, germinação in vitro de pólen, melhoramento de plantas, tubo polínico.

Genipap (*Genipa americana* L.), belonging to the Rubiaceae family, is a native and non-endemic species to Brazil, occurring in all five regions of the country (Gomes, 2022). This species was listed among the top ten native fruit trees with the greatest potential for immediate use in Brazil according to the "Plantas do Futuro" program, developed, in partnership, by Conselho Nacional de Desenvolvimento Científico e Tecnológico/World Bank/Global Environment Facility/Ministério do Meio Ambiente/Projeto de Conservação e Utilização Sustentável da Diversidade Biológica Brasileira (Coradin et al., 2018).

To guarantee the maintenance of genetic variability in a species, it is important to evaluate the viability of pollen, whose grains carry genetic material resulting from recombination, increasing the likelihood of the

plants transmitting highly diverse genotypes to the next generation (Zortéa et al., 2022). Pollen viability can be determined by several methods, such as in vitro and in vivo pollen tube growth and histochemical staining using different dyes (Impe et al., 2020). Among the methods for testing pollen viability, in vitro pollen germination is considered one of the most convenient and reliable as it indicates the ability of pollen grains to emit the pollen tube (Luo et al., 2020).

For in vitro germination, each species has specific requirements regarding the composition of the used culture medium due to genetic variations and pollen grain osmotic pressure (Lin et al., 2017). For a satisfactory germination and tube growth, generally moisture, a carbohydrate source, boron, and calcium are required (Patel & Mankad, 2014).

In the case of genipap, further research is necessary, particularly related to floral biology, ex situ conservation, and cryopreservation for future ex situ conservation and pollination studies. In addition, up to date, there is no known information available on the in vitro pollen germination of the species.

The objective of this work was to evaluate the viability of genipap pollen grain at room temperature in different culture media.

For the experiment, 20 functional male flowers were collected from a natural population found in the municipality of Siriri, in the state of Sergipe, Brazil (10°60'30"S, 37°11'28"W), at the pre-anthesis stage, i.e., 24 hours before opening, between 9:00 and 10:00 a.m. After the pedicel was cut off, the flowers were conditioned in a tightly closed paper bag and maintained in expanded polystyrene boxes, which were taken to the Laboratory of Plant Tissue Culture of Embrapa Tabuleiros Costeiros, located in the municipality of Aracaju, in the same state. At the laboratory, fine-nose forceps were used to extract pollen grains from the anthers opened on aluminum foil.

To evaluate the effect of different culture media on in vitro germination, approximately 0.0005 g pollen grains were inoculated on 10x35 mm sterile petri dishes, containing 2.0 mL of the three following culture media: A, 200 mg L^{-1} $MgSO_4.7H_2O$, 300 mg L^{-1} $Ca(NO_3)O_2.4H_2O$, 100 mg L^{-1} KNO_3, 100 mg L^{-1} H_3BO_3, and 40 g L^{-1} sucrose as in Lora et al. (2006); B, 100 g L^{-1} sucrose and 3.0 g L^{-1} agar according to Sousa et al. (2010); and C, 100 g L^{-1} H_3BO_3, 80 g L^{-1} sucrose, and 1.0 g L^{-1} agar, following Sousa et al. (2010) with modifications by Moura et al. (2015). For this analysis, each culture medium was spread on 11 petri dishes with four compartments (separate counting fields) each and maintained in a biological incubator at 27±2ºC. The number of germinated pollen grains was analyzed under a 10X microscope objective at 24-hour intervals up to 288 hours after incubation. The total number of pollen grains and number of pollen grains germinated in each compartment were counted under the microscope. To calculate the percentage of in vitro pollen grain germination, the following formula was used: in vitro pollen grain germination (%) = (number of pollen grains germinated / total number of counted pollen grains) x 100.

The experimental design was completely randomized in a 3x11 factorial arrangement (culture media x incubation times) with four replicates, each composed of one Petri dish with the four counting fields. For the statistical analysis, data of in vitro pollen grain (%) were analyzed by the analysis of variance using the F-test. For the qualitative factor (culture media), means were compared by Tukey's test, at 5% probability. For the quantitative factor (pollen grain incubation times), regression equations were estimated. All analyses were conducted using the SISVAR software (Ferreira, 2019).

In vitro pollen grain germination differed significantly by the F-test due to the interaction between different culture media and incubation times (Table 1). Despite this, all culture media showed favorable nutritional conditions for pollen tube development. From 192 to 288 hours of incubation, there was no significant difference in pollen germination among culture media B and C; the absence of boron and calcium in the former did not affect pollen tube emission. The exception was medium A, which showed a drastic reduction in germination percentage at 288 hours. Similar results were reported by Souza et al. (2021) for pollen germination of *Coffea canephora* Pierre ex A.Froehner (Rubiaceae). Genotype variation in family Rubiaceae was observed by comparing the results obtained for pollen grains of its species with those of others that also require boron and calcium for in vitro pollen grain germination, such as Acauã coffee (Angelo, 2015), *Hamelia patens* Jacq. (Verma et al., 2017), and *Ixora coccinea* L. (Phanomchai et al., 2021).

Table 1. In vitro pollen grain germination of genipap (*Genipa americana*) at room temperature (27±2ºC) as a function of culture media and incubation time at 24-hour intervals up to 288 hours after incubation[1].

Culture media[2]	In vitro pollen grain germination (%)										Equation
	24	48	72	120	144	168	192	216	240	264	288	
A	52.19B	95.48A	92.46A	96.91A	72.79A	76.64A	75.36A	77.77A	70.10A	52.56A	20.04B	$Y = -0.002^{**}x^2 + 0.559^{**}x + 6.04^{**}$; $R^2 = 0.737$
B	85.88A	76.49B	83.30A	86.60AB	82.40A	55.57B	69.96A	70.94A	60.89A	42.94A	54.97A	$Y = 0.15^{**}x - 91.66^{**}$; $R^2 = 0.673$
C	89.24A	87.64AB	81.74A	71.75B	53.51B	67.99AB	69.24A	70.55A	73.04A	59.02A	56.60A	$Y = -0.000011x3 + 0.00052^{*}x^2 - 0.084^{**}x + 111.75^{**}$; $R^2 = 0.652$
CV (%)	12.88											

[1]Means followed by equal letters, in the lines, differ from each other by Tukey's test, at 5% probability. [2]Culture media A, B, and C according to Lora et al. (2006), Sousa et al. (2010), and Sousa et al. (2010) modified by Moura et al. (2015), respectively. CV, coefficient of variation. ** and *Significant by the regression coefficient at 1 and 5% probability, respectively. [ns]Nonsignificant.

For incubation times, medium A followed a quadratic model, with an optimal time and maximum germination of 139.75 hours and 95.10%, respectively. Medium B presented a negative linear regression, in which germination percentage decreased from 86.60 to 42.94%; however, it still showed a high to intermediate pollen viability. Culture medium C resulted in a cubic equation, exhibiting a high to intermediate pollen germination percentage, with constant viability values. Likewise, Souza et al. (2021) found pollen viability from 40 to 90% for different *C. canephora* genotypes.

The obtained results are indicative that medium C presented adequate nutritional conditions for pollen tube growth, being the most suitable for genipap in vitro pollen grain germination. The observed findings contribute towards genipap species conservation and genetic breeding programs.

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