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In vitro germination of passion fruit seeds in the function of tegument removal and culture media[1]

Germinação in vitro de sementes de maracujá em função da remoção do tegumento e meios de cultura

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HIGHLIGHTS:

Low germination can be related to tegument impermeability and mechanical techniques allowing imbibition must be employed.
The use of gibberellins increases germination percentage.
The stress induced by salts in culture media decreases the water potential and germination.

ABSTRACT: The presence of salts in the culture medium and the addition of phytohormones, necessary to break seed dormancy in the in vitro germination of passion fruit, can induce critical values of osmotic potential. This can change the membrane permeability of seeds, affecting the hydration rate, enzyme release, ion transport, pH, and inhibitor values, which may decrease germination percentage. The aim of this study was to evaluate different types of culture media, composed of other substances, combined with mechanical techniques of tegument removal to determine the most appropriate culture medium for the in vitro germination of *Passiflora edulis* (*Sims flavicarpa Deg.*) seeds obtained by open pollination. The experimental design was completely randomized in a 10 × 3 factorial scheme (10 culture media × three types of seed-intact, scarified, and cut), with eight replicates and five seeds per plot. Scarified seeds cultivated in a medium composed of a commercial substrate and gibberellic acid presented a higher percentage of germination than intact or cut seeds cultivated in different concentrations of Murashige & Skoog (MS) medium. Scarification is a satisfactory method for breaking the dormancy of passion fruit seeds, and commercial substrates Bioflora® enriched with gibberellic acid may replace in vitro germination.

Key words: *Passiflora edulis* (*Sims flavicarpa Deg.*), tissue culture, abiotic stress, seeds viability, non-parametric tests

RESUMO: A presença de sais no meio de cultura e a adição de fitohormônios, necessários para quebrar a dormência das sementes na germinação in vitro do maracujá, podem levar o potencial osmótico a níveis críticos. Isso pode alterar a permeabilidade da membrana das sementes, afetando a taxa de hidratação, a liberação de enzimas, o transporte iônico, o pH e os teores de inibidores, o que pode acarretar na diminuição do percentual de germinação. O objetivo desta pesquisa foi avaliar diversos tipos de meios de cultura, compostos por diferentes substâncias, combinados com técnicas mecânicas de remoção do tegumento, para determinar o tratamento mais adequado para a germinação in vitro de sementes de *Passiflora edulis* (*Sims flavicarpa Deg.*) obtidas por polinização aberta. O delineamento experimental foi inteiramente casualizado, em esquema fatorial 10 × 3 (10 meios de cultura × três tipos de sementes - intactas, escarificadas e cortadas), com oito repetições e cinco sementes por parcela. Sementes escarificadas cultivadas em meio composto de substrato comercial e ácido giberélico apresentaram taxa de germinação superior às sementes intactas ou cortadas cultivadas em diferentes concentrações de meio Murashige & Skoog (MS). A escarificação é um método satisfatório de quebra de dormência de sementes de maracujá e substratos comerciais Bioflora® enriquecidos com ácido giberélico podem substituir o meio MS na germinação in vitro.

Palavras-chave: *Passiflora edulis* (*Sims flavicarpa Deg.*), cultura de tecidos, estresse abiótico, viabilidade de sementes, testes não paramétricos

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Introduction

Passion fruit has become an important export product for South America, yielding approximately 1.2 million dollars in the Brazilian market in 2019 (Comex Stat, 2019). However, crops suffer from phytosanitary problems affecting plant life cycles, productivity, and fruit quality. *Passiflora edulis* is highly susceptible to soil-related diseases and bacterial contamination (Faleiro et al., 2019).

In vitro germination techniques can be valuable tools for researchers, as aseptic explants are generated with the advantage of offering a better morphogenic response when compared to adult tissues, producing reliable seedlings, especially considering species with dormancy or germination issues, with a high incidence of pathogens during seed storage (Merkle et al., 2022). These techniques allow direct organogenic multiplication, juvenility, and greater variability in the same batch, thus improving the quality and development of the plant (Faria et al., 2020).

To achieve successful in vitro germination, abiotic stress must induce dormancy breaks using chemical and/or physical methods (Grzybowski et al., 2019; Jasper & Nyamweha, 2019; Saffari et al., 2021). The choice of appropriate culture media is also essential because different concentrations of salts can positively or negatively influence the germination process depending on the osmotic potential of seeds (Uçarlı, 2020), while the addition of enriching agents and growth regulators may be necessary for the success of germination (Miranda et al., 2018; Uçarlı, 2020).

The aim of this study was to evaluate different types of culture media, composed of different substances, combined with mechanical techniques of tegument removal, to determine the most appropriate for the in vitro germination of *Passiflora edulis* (*Sims flavicarpa Deg.*) seeds obtained by open pollination.

Material and Methods

Experiments were performed in March 2018, at the Laboratório de Cultura de Tecidos Vegetais (LCTV) of the Departamento de Fitotecnia, Tecnologia de Alimentos e Sócio Economia, Universidade Estadual Paulista - UNESP (Ilha Solteira, SP, Brazil), 20° 25' 24.4" South latitude and 51° 21' 13.1" West longitude, with an altitude around 337 m.

The seeds were removed from the fruits and placed in an inert container for 24 hours for natural fermentation. They were then placed in a steel sieve (1 mm) and washed in ultrapure (deionized and distilled) running water for aril removal. Afterward, they were dried for 48 hours on absorbent paper at ± 28 °C.

Morphometric analyses were performed, and the following variables were evaluated: mass (MA, in mg), thickness (TH, in mm), width (WI, in mm), and length (LE, in mm). After the measurement, a descriptive data analysis was conducted, and seeds were separated for germination based on the obtained inferences. Nontrivial statistical techniques were applied to respect the nature of the qualitative and quantitative variables.

Selected seeds were then disinfected in 70 % (v/v) ethyl alcohol for 1 minute and then in a solution of 2.5% sodium hypochlorite with two drops of Tween 20 (Dynamic®) for 30 min. Triple rinsing was performed in a laminar flow cabinet using ultrapure sterile water. The seeds randomly distributed among the culture media were selected from those within the interquartile range. That is, 50% of the evaluated seeds were selected based on the four observed variables to achieve greater homogeneity in germination.

Some selected seeds were subjected to mechanical tegument removal techniques such as scarification (using sandpaper 150) and cutting (both in the opposite region and emission of the radicle, using a surgical scalpel). The seeds were divided into three groups: intact, scarified, and cut (Figure 1).

Seeds were inoculated in glass vials (88 × 64 mm) containing the following culture media (treatments - T): (T1): commercial organic substrate (Bioflora®) + 40 mL water; (T2): commercial organic substrate (Bioflora®) + 40 mL nutrient solution of Murashige & Skoog (MS) medium (Murashige & Skoog, 1962) with 50% salts; (T3): commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 100% salts; (T4): MS medium with 50% salts, 30 g L^{-1} sucrose, pH adjusted to 5.7 ± 0.3 and solidified with 5 g L^{-1} agar; (T5): MS medium with 100% salts, 30 g L^{-1} sucrose, pH adjusted at 5.7 ± 0.3 and solidified with 5 g L^{-1} agar; (T6): commercial organic substrate (Bioflora®) + 40 mL solution containing 75 mg L^{-1} gibberellic acid (GA3) (Dynamic®); (T7): commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 50% salts + 75 mg L^{-1} GA3; (T8): commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 100% salts + 75 mg L^{-1} GA3; (T9): MS medium with 50% salts, 30 g L^{-1} sucrose, pH adjusted to 5.7 ± 0.3 and solidified with 5 g L^{-1} agar + 75 mg L^{-1} GA3; and (T10): MS medium with 100% salts, 30 g L^{-1} sucrose, pH adjusted to 5.7 ± 0.3 and solidified with 5 g L^{-1} agar + 75 mg L^{-1} GA3.

Substrates and culture media were sterilized in an autoclave for 20 minutes at 1 atm and 121 °C and cooled to room temperature before inoculation.

After inoculation, the vials were placed in a BOD incubator at 28 °C in the dark for 40 days. After this period, the plants were transferred to a growth chamber with a photoperiod of 16 hours at 25 ± 3 °C and active photosynthetic radiation of 45-55 µmol m^{-2} s^{-1}.

The experimental design was completely randomized in a 10 × 3 factorial scheme (10 culture media × three types of seed-intact, scarified, and cut), with eight replicates and five seeds per plot.

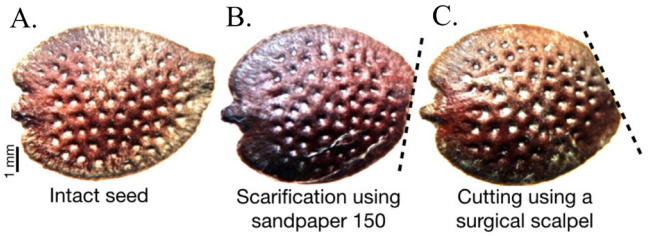

Figure 1. Passion fruit seeds - intact (A), scarified (B), and cut (C) in the region opposite the radicle emission

Forty days after inoculation, the percentages of exogenous and endogenous contamination and germination were assessed for each vial. Data were statistically evaluated using non-parametric chi-square and Fisher's exact tests to analyze the associations between qualitative variables. When one of the cells presented a frequency < 5, Fisher's exact test was applied at $p \leq 0.05$.

For germination (%), data were submitted to analysis of variance by the F test, and the comparison of means was performed by the Scott-Knott (culture media) at $p \leq 0.05$ using software R (R Development Core Team, 2022).

Results and Discussion

According to descriptive statistics, passion fruit seeds presented mean values of the mass of 22.01 mg, whereas thickness, width, and length presented mean values of 1.91, 4.07, and 5.89 mm, respectively (Table 1).

The interquartile range IQ (quartile 3 - quartile 1) of variable MA was of 2.55 (23.55 - 21.00), for TH 0.30 (2.12 - 1.82), for WI 0.27 (4.23 -3.96), and for LE 0.34 (6.06 - 5.72). The highest variability was recorded in TH, with a coefficient of variation (CV) of 14.98%, indicating a greater dispersion of data, followed by WI (10.86%), LE (7.29%), and TH (5.91%). The variables MA and LE presented symmetry, whereas the other variables presented weak asymmetry. Two outliers were observed for MA and WI and three for LE, which affected the mean of the data. However, this did not influence the interquartile range and, consequently, did not interfere in this study (Figure 2). According to Silveira et al. (2019), variations in the morphological characteristics of seeds are mainly related to gene expression and genetic variability in populations of Passiflora associated with local environmental conditions.

The contamination percentage was high, with 33% contamination of intact seeds, 69% of scarified seeds, and 72% of cut seeds. However, according to Fisher's exact test, this contamination was not influenced by culture media or methods of tegument removal and was probably due to endogenous factors (Table 2).

For intact seeds, all culture media presented a p-value of 1. In all the culture media, the value of endogenous contamination was the same, reaching 33%. Scarified seeds presented a p-value of 0.58 for all culture media, and results were not statistically significant, in which the endogenous contamination reached 69%.

Table 1. Descriptive statistics of mass (MA), thickness (TH), width (WI), and length (LE) of passion fruit seeds

Descriptive statistics	MA (mg)	TH (mm)	WI (mm)	LE (mm)
Minimal	15.800	0.018	3.470	5.060
First quartile	23.550	2.115	4.225	6.060
Median	21.900	1.960	4.110	5.910
Arithmetic mean	22.018	1.919	4.075	5.898
Third quartile	21.000	1.815	3.960	5.720
Maximal	26.100	2.220	4.630	6.670
Standard deviation	2.324	0.288	0.241	0.315
Standard error	0.232	0.029	0.024	0.032
CV (%)	10.554	14.985	5.925	5.343

CV - Coefficient of variation

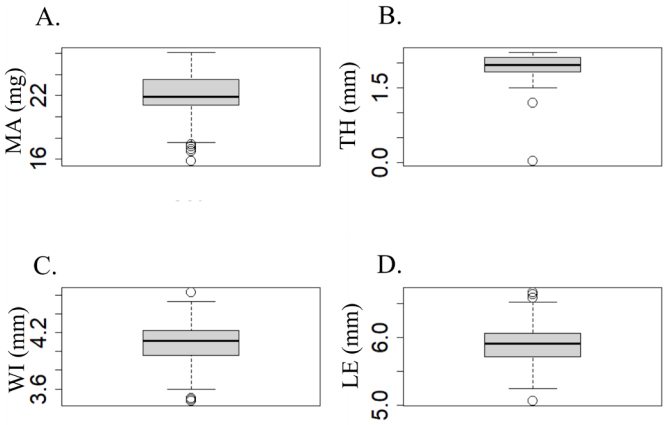

Figure 2. Boxplot for variables mass (MA), thickness (TH), width (WI), and length (LE) of passion fruit seeds selected for the experiments

Table 2. Fisher's exact test p values for endogenous contamination of passion fruit seeds in different culture media

Culture media	T2	T3	T4	T5	T6	T7	T8	T9	T10
Intact seeds									
T1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
T2		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
T3			1.00	1.00	1.00	1.00	1.00	1.00	1.00
T4				1.00	1.00	1.00	1.00	1.00	1.00
T5					1.00	1.00	1.00	1.00	1.00
T6						1.00	1.00	1.00	1.00
T7							1.00	1.00	1.00
T8								1.00	1.00
T9									1.00
Scarified seeds									
T1	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
T2		0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
T3			0.58	0.58	0.58	0.58	0.58	0.58	0.58
T4				0.58	0.58	0.58	0.58	0.58	0.58
T5					0.58	0.58	0.58	0.58	0.58
T6						0.58	0.58	0.58	0.58
T7							0.58	0.58	0.58
T8								0.58	0.58
T9									0.58
Cut seeds									
T1	1.00	1.00	0.29	0.58	1.00	1.00	1.00	1.00	0.29
T2		1.00	0.29	0.58	1.00	1.00	1.00	1.00	0.29
T3			0.29	0.58	1.00	1.00	1.00	1.00	0.29
T4				1.00	0.29	0.29	0.57	0.57	1.00
T5					0.58	0.58	1.00	1.00	1.00
T6						1.00	1.00	1.00	0.29
T7							1.00	1.00	0.29
T8								1.00	0.57
T9									0.57

T1 - Commercial organic substrate (Bioflora®) + 40 mL water; T2 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium (Murashige & Skoog, 1962) with 50% salts; T3 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 100% salts; T4 - MS medium with 50% salts, 30 g L^{-1} sucrose, pH adjusted to 5.7 ± 0.3 and solidified with 5 g L^{-1} agar; T5 - MS medium with 100% salts, 30 g L^{-1} sucrose, pH adjusted at 5.7 ± 0.3 and solidified with 5 g L^{-1} agar; T6 - Commercial organic substrate (Bioflora®) + 40 mL solution containing 75 mg L^{-1} gibberellic acid (GA3) (Dynamic®); T7 - commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 50% salts + 75 mg L^{-1} GA3; T8 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 100% salts + 75 mg L^{-1} GA3; T9 - MS medium with 50% salts, 30 g L^{-1} sucrose, pH adjusted to 5.7 ± 0.3 and solidified with 5 g L^{-1} agar + 75 mg L^{-1} GA3; T10 - MS medium with 100% salts, 30 g L^{-1} sucrose

Variations between the culture media were observed for the cut seeds, with an amplitude of 71%. The p-value ranged from 0.29 to 1.00 but did not differ statistically.

Results related to endogenous contamination suggest that contamination occurred before inoculation, probably because

of the lack of phytopathological control of the seeds derived from open pollination.

Table 3 presents the statistical results for the exogenous contamination. Regarding exogenous contamination, culture medium T4 statistically differed from culture media T1, T6, T7, and T8; T5 differed from T6, T7, and T8; T6 differed from T9; T7 differed from T9 and T10; and T8 differed from T9 and T10, according to Fisher's test with a probability of 0.05.

Concerning exogenous contamination on the germination of cut seeds, T4 presented a p-value of 0.05, differing from T1, T7, and T8 according to Fisher's test. When considering p-values of 0.02, statistical differences between T9, T1, T3, T6, T7, and T8 were obtained.

Statistical differences related to the number of germinated plots among intact seeds and significant differences were obtained for T5 and T1 and T6, T7, and T8 (0.00 - 0.01); for T9 and T3, and T2, T3, T1, T7, and T8 with p-values of 0.02 (Table 4).

Before inoculation, glass vials, substrates, and culture media were sterilized using an autoclave, while seeds were disinfected with sodium hypochlorite and ethyl alcohol 70%. Sterilization of the culture medium and glassware before inoculation is essential for disinfection and preventing exogenous contamination during germination and development of seedlings (Faria et al. 2020). Regarding the disinfection of biological materials, a combination of sodium hypochlorite and ethyl alcohol is among the most frequently used agents for sterilization. However, contamination still occurred in the present study despite the disinfection of the seeds and sterilization of the material. Song et al. (2022) suggested that these agents may fail to remove contaminants efficiently depending on the circumstances of the sampling and storage of biological materials. The contamination observed in this study may be due to the origin of passion fruits used and reinforces the need for quality matrices for satisfactory in vitro germination.

For the different seeds and culture media, $p \leq 0.01$ were found, regarding the interaction, it was verified significance at $p \leq 0.05$ (Table 5).

The germination percentages were low and did not reach 50% for all treatments. For intact seeds, the highest germination percentage was recorded in T9, while for cut seeds,

Table 3. Fisher's exact test p-values for exogenous contamination of passion fruit seeds

Culture media	T2	T3	T4	T5	T6	T7	T8	T9	T10
Intact seeds									
T1	1.00	1.00	0.02	1.00	1.00	1.00	1.00	1.00	1.00
T2		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
T3			1.00	1.00	1.00	1.00	1.00	1.00	1.00
T4				1.00	0.02	0.02	0.02	1.00	1.00
T5					0.02	0.02	0.02	1.00	1.00
T6						1.00	1.00	0.02	0.02
T7							1.00	0.02	0.02
T8								0.02	0.02
T9									1.00
Scarified seeds									
T1	1.00	1.00	0.00	1.00	1.00	1.00	1.00	0.02	1.00
T2		1.00	1.00	1.00	1.00	1.00	1.00	0.02	1.00
T3			1.00	1.00	1.00	1.00	1.00	0.02	1.00
T4				1.00	0.00	0.00	0.00	0.07	1.00
T5					1.00	1.00	1.00	1.00	1.00
T6						1.00	1.00	0.02	1.00
T7							1.00	0.02	1.00
T8								0.02	1.00
T9									1.00
Cut seeds									
T1	1.00	1.00	0.13	0.05	0.13	0.13	0.13	0.02	0.13
T2		1.00	1.00	1.00	1.00	1.00	1.00	0.02	1.00
T3			1.00	1.00	1.00	1.00	1.00	0.02	1.00
T4				1.00	1.00	1.00	1.00	1.00	1.00
T5					0.05	0.05	0.05	1.00	1.00
T6						1.00	1.00	0.02	1.00
T7							1.00	0.02	1.00
T8								0.02	1.00
T9									0.61

T1 - Commercial organic substrate (Bioflora®) + 40 mL water; T2 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium (Murashige & Skoog, 1962) with 50% salts; T3 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 100% salts; T4 - MS medium with 50% salts, 30 g L^{-1} sucrose, pH adjusted to 5.7 ± 0.3 and solidified with 5 g L^{-1} agar; T5 - MS medium with 100% salts, 30 g L^{-1} sucrose, pH adjusted at 5.7 ± 0.3 and solidified with 5 g L^{-1} agar; T6 - Commercial organic substrate (Bioflora®) + 40 mL solution containing 75 mg L^{-1} gibberellic acid (GA3) (Dynamic®); T7 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 50% salts + 75 mg L^{-1} GA3; T8 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 100% salts + 75 mg L^{-1} GA3; T9 - MS medium with 50% salts, 30 g L^{-1} sucrose, pH adjusted to 5.7 ± 0.3 and solidified with 5 g L^{-1} agar + 75 mg L^{-1} GA3; T10 - MS medium with 100% salts, 30 g L^{-1} sucrose

Table 4. Fisher's exact test p values for the number of germinated plots of sour passion fruit seeds in culture media

Culture media	T2	T3	T4	T5	T6	T7	T8	T9	T10
Intact seeds									
T1	1.00	1.00	0.00	0.05	1.00	1.00	1.00	0.02	0.00
T2		1.00	0.00	0.05	1.00	1.00	1.00	0.02	0.00
T3			0.00	0.05	1.00	1.00	1.00	0.02	0.00
T4				0.50	0.00	0.00	0.00	1.00	1.00
T5					0.05	0.05	0.05	1.00	0.58
T6						1.00	1.00	0.02	0.00
T7							1.00	0.02	0.00
T8								0.02	0.00
T9									1.00
Scarified Seeds									
T1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.58	0.29
T2		1.00	1.00	1.00	1.00	1.00	1.00	0.58	0.29
T3			1.00	1.00	1.00	1.00	1.00	0.58	0.29
T4				1.00	1.00	1.00	1.00	1.00	0.57
T5					1.00	1.00	1.00	1.00	0.57
T6						1.00	1.00	0.58	0.29
T7							1.00	1.00	0.57
T8								1.00	0.57
T9									1.00
Cut Seeds									
T1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
T2		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
T3			1.00	1.00	1.00	1.00	1.00	1.00	1.00
T4				1.00	1.00	1.00	1.00	1.00	1.00
T5					1.00	1.00	1.00	1.00	1.00
T6						1.00	1.00	1.00	1.00
T7							1.00	1.00	1.00
T8								1.00	1.00
T9									1.00

T1 - Commercial organic substrate (Bioflora®) + 40 mL water; T2 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium (Murashige & Skoog, 1962) with 50% salts; T3 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 100% salts; T4 - MS medium with 50% salts, 30 g L^{-1} sucrose, pH adjusted to 5.7 ± 0.3 and solidified with 5 g L^{-1} agar; T5 - MS medium with 100% salts, 30 g L^{-1} sucrose, pH adjusted at 5.7 ± 0.3 and solidified with 5 g L^{-1} agar; T6 - Commercial organic substrate (Bioflora®) + 40 mL solution containing 75 mg L^{-1} gibberellic acid (GA3) (Dynamic®); T7 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 50% salts + 75 mg L^{-1} GA3; T8 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 100% salts + 75 mg L^{-1} GA3; T9 - MS medium with 50% salts, 30 g L^{-1} sucrose, pH adjusted to 5.7 ± 0.3 and solidified with 5 g L^{-1} agar + 75 mg L^{-1} GA3; T10 - MS medium with 100% salts, 30 g L^{-1} sucrose

Table 5. Summary of analysis of variance and means of germination of intact scarified and cut passion fruit seeds cultivated in different treatments

Methods of tegument removal (TR)	Culture media (T)	TR × T	CV (%)
≤ 0.01**	≤ 0.01**	0.0140*	33.63
Culture media	Germination (%)		
	Intact seeds	Scarified seeds	Cut seeds
1	0.00 bA*	0.00 cA	0.00 bA
2	2.50 bA	5.00 cA	2.50 bA
3	2.50 bA	5.00 cA	2.50 bA
4	2.50 bA	5.00 cA	2.50 bA
5	2.50 bA	5.00 cA	2.50 bA
6	2.50 bC	47.5 aA	27.50 aB
7	2.50 bB	27.50 bA	2.50 bB
8	2.50 bA	5.00 cA	2.50 bA
9	27.50 aA	7.50 cB	5.00 bB
10	2.50 bB	27.50 bA	2.50 bB

** - Significant at 0.01; * - significant at 0.05 probability by F test
* Mean values with the same capital letter between columns and lower case letter between rows do not differ from each other by the Scott-Knott test at $p \leq 0.05$
T1 - Commercial organic substrate (Bioflora®) + 40 mL water; T2 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium (Murashige & Skoog, 1962) with 50% salts; T3 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 100% salts; T4 - MS medium with 50% salts, 30 g L^{-1} sucrose, pH adjusted to 5.7 ± 0.3 and solidified with 5 g L^{-1} agar; T5 - MS medium with 100% salts, 30 g L^{-1} sucrose, pH adjusted at 5.7 ± 0.3 and solidified with 5 g L^{-1} agar; T6 - commercial organic substrate (Bioflora®) + 40 mL solution containing 75 mg L^{-1} gibberellic acid (GA3) (Dynamic®); T7 - commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 50% salts + 75 mg L^{-1} GA3; T8 - Commercial organic substrate (Bioflora®) + 40 mL nutrient solution of MS medium with 100% salts + 75 mg L^{-1} GA3; T9 - MS medium with 50% salts, 30 g L^{-1} sucrose, pH adjusted to 5.7 ± 0.3 and solidified with 5 g L^{-1} agar + 75 mg L^{-1} GA3; T10 - MS medium with 100% salts, 30 g L^{-1} sucrose

it was recorded in T6 (both 27.5%). Scarified seeds presented a higher rate of germination among all seeds, with germination percentages of 5% (T2 - T5 and T8), 7.5% (T9), 27.5% (T7 and T10), and 47.5% (T6) (Table 4). Intact, scarified, and cut seeds did not germinate in T1. Overall, higher percentages were recorded for T6, T7, T9 and T10, among all types of seeds.

According to Castilho et al. (2020), damage to the seed tegument, endosperm, and embryo negatively affects water uptake; therefore, all early stages of germination are altered. This fact can be verified in the present study, as cut seeds had a lower germination rate than scarified seeds. Angelini et al. (2021) also obtained higher germination percentages for dry seeds scarified at their tips in *P. incarnata*.

Despite being composed of different substances, T6, T7, T9, and T10 contained gibberellic acid in their composition. The highest germination percentage was recorded for scarified seeds cultivated in T6, which was composed of a mixture of commercial organic substrate, water, and gibbelleric acid. Thus, it is possible to affirm that the addition of gibberellin demonstrated positive effects, corroborating the results reported by other authors for passion fruit seeds (Grzybowski et al., 2019). The hormone gibberellin plays a role in the synthesis of RNAs and proteins responsible for seed germination, regulating the synthesis of α- and β-amylase enzymes responsible for the hydrolysis of seed reserve tissues, transforming reserves into amino acids, nucleic acids, and sugars, and displacing these compounds into regions of embryonic growth.

Treatment 9 (T9) was the second most suitable for inducing germination, considering all types of seeds, with germination rates of 27.5, 7.5%, and 5% of intact, scarified, and cut seeds, respectively. This treatment consisted of a mixture of MS 50%, sucrose, and gibberellic acid.

Faria et al. (2020) also found positive effects of MS medium 50% for the in vitro germination of passion fruit seeds when compared to pure MS medium. In this study, the higher concentrations of salts found in pure MS presumably promoted a decrease in water intake by the seeds, negatively interfering with the germination process. On the other hand, the reduction of salts led to better plant development, probably because of the passion fruit's sensitivity to salinity (Ramos et al., 2022). Moreover, the use of half-concentrated medium is economically advantageous because less material is used to achieve germination success (Faria et al., 2020).

Although very important, the culture medium is not the only factor influencing germination success. Some species of the genus *Passiflora* spp. contain seeds that are dormant because of physical, chemical, or physiological factors, such as tegument impermeability to water or resistance to embryo growth, which can negatively affect germination success (Alves-Junior et al., 2020). Thus, in in vitro cultivation, effective techniques for breaking the dormancy of seeds are necessary. Removal of the aril, for example, by natural fermentation, chemical, and/or mechanical techniques may stop dormancy and accelerate germination (Grzybowski et al., 2019). Gibberellic acid also accelerates seed dormancy, breaking and standardizing the germination process (Santos et al., 2022).

Considering the mechanical techniques of tegument removal applied in this study, scarified seeds presented the highest rates of germination (13.75%), regardless of the treatment, when compared to intact (4.75%) and cut (5.00%) seeds (Table 4), suggesting that this method was more efficient in breaking dormancy, possibly facilitating water permeability into the seeds (Angelini et al., 2021). Water penetration via the tegument and consequent contact with the embryonic axis may accelerate the germinative processes, due to an increase in the respiratory rate of tissues and other activities in the seed metabolism, which results in the supply of nutrients and energy for the return of embryonic growth (Corrêa et al., 2022). Low germination values for intact seeds may be related to the inefficiency of dormancy breaking uniquely by seed fermentation and acidification, which might prevent water from penetrating the seeds. However, cutting the seeds might have damaged the embryonic axis or reserve tissues, impairing germination.

Overall, it was possible to verify that the commercial substrate was efficient for the germination of scarified passion fruit seeds when combined with gibberellic acid, despite the high endogenous seed contamination. These findings can be useful because commercial substrates are usually less expensive than MS medium, and cultivation can be performed without laboratory conditions. If combined with seeds originating from quality matrices, these methods may be suitable for the cultivation of passion fruit, with high success rates of germination and low costs.

Conclusions

1. Commercial substrates enriched with gibberellin acid may be used to replace culture media, such as Murashige & Skoog (MS), for in vitro germination.

2. Scarification showed to be a satisfactory method of germination of passion fruit seeds.

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