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Effect of pollination on the viability of passion fruit seeds

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ABSTRACT. Seed propagation is the most used method for producing yellow passion fruit seedlings (*Passiflora edulis* f. *flavicarpa*); however, the success and uniformity of seedling formation are influenced by the germination process. This study aims to evaluate the influence of light and different pollination conditions on the quality of passion fruit seeds and seedlings. The experiment was conducted in two stages, both arranged in a completely randomized design (CRD): In the first stage, two treatments were used to determine the optimal light conditions for the germination of *Passiflora edulis* seeds obtained from fruits formed through manual cross-pollination (MCP). In the second stage, the effects of different pollination conditions on seed germination and seedling development were evaluated. The variables analyzed included germination rate, mean germination time, germination speed index, total seedling length, shoot length, root length, and fresh and dry biomass. The results indicated that the absence of light promotes a higher germination rate and a shorter mean germination time for *P. edulis* seeds. Flowers subjected to manual cross-pollination (MCP) and free visitation without pollen thieves (FVWT) resulted in higher germination rates and shorter germination times. The presence of thieves caused a 31% reduction in seed germination but did not affect seedling development.

Keywords: bees, germination, *Passiflora edulis*.

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Introduction

The yellow passion fruit (*Passiflora edulis* f. *flavicarpa*) is predominantly propagated through seeds (José et al., 2019); however, seed production by this method often yields unsatisfactory results due to the low seed germination percentage and poor emergence uniformity, a common characteristic among species of the *Passiflora* genus, attributed to the temporary dormancy exhibited shortly after harvest (Meletti et al., 2002).

The success and uniformity of seedling development largely depend on the quality of the germination process, making it essential to understand the germination constraints of the species. These constraints include genetics (differences among species), management (phytosanitary issues, climate variability, drying, storage), and morphology and physiology (dormancy, maturity, vigor, photoblastism) (Marcos-Filho, 2005; Alvarenga, 2013; Silva et al., 2013).

Pollination also plays a critical role in fruit and seed formation and, when carried out effectively, can contribute to improving seed physiological quality (Nascimento et al., 2012). In this context, successful fruit set, fruit diameter, and fruit quality depend on ovule fertilization and ovary filling (Ariizumi et al., 2013). Poor pollination can result in fruit deformities, low fruit set, and reduced yield, which are directly linked to pollen availability and the presence of pollinating insects in the cultivation area (Nascimento et al., 2012).

Several studies have evaluated the effect of natural pollination carried out by bees on fruit and seed formation. For instance, onion crops require pollinating insects such as bees and house flies for efficient pollination and consequent seed production (Witter & Blochtein, 2003). The carrot, an allogamous plant, requires pollinating insects to produce seeds (Castellane, 1980). Bell pepper, despite being an autogamous plant, benefits from bee pollination, producing significantly heavier and wider fruits, in addition to increasing seed production and quality (Cardoso, 2007). There are also positive reports in eggplant (Nunes-Silva et al., 2013), Suriname cherry (Fidalgo et al., 2018), pepper (Nascimento et al., 2012) and okra (Carvalho et al., 2020).

Thus, considering that germination is a physiological phenomenon influenced by several factors, the present work aims to evaluate the effect of different floral visiting conditions on the viability of passion fruit seeds and seedlings.

Material and methods

The experiment was carried out from August to October 2021 in the Laboratory of Semiarid Biodiversity (LABISA), at the State University of Southwestern Bahia, campus Vitória da Conquista, Bahia state, Brazil,

Fruits were obtained from a commercial yellow passion fruit field (*P. edulis* f. *flavicarpa* – 'BRS Gigante Amarelo' cultivar), located in the municipality of Barra do Choça – Bahia state, Brazil (14°48'52" S latitude and 40°31'3" W longitude). Flowers were subjected to different pollination conditions (30 flowers per treatment) and monitored throughout their receptivity period during the day. The treatments included: Monitored Free Visitation (MFV), Manual Cross-Pollination (MCP), and Free Visitation Without Pollen Thieves (FVWT), in which visits by *Apis mellifera* and *Trigona spinipes* were manually excluded. The fruits meeting commercial standards were harvested at full physiological maturity in the field and transported to the laboratory for seed viability assessment.

Initially, the seeds were extracted from the fruits, washed under running water, and placed on film paper in the shade at room temperature for 24 hours to dry. Then, the aril was manually removed, following the method described by Carvalho and Nakagawa (2000). The effectiveness of aril removal is closely associated with the attainment of high-quality seeds, and the choice of method depends on fruit characteristics, the degree of aril adhesion, and the presence of the gelatinous envelope surrounding the seed (Silva, 2000). After aril removal, the seeds were disinfected in a 2% sodium hypochlorite solution for 2 minutes and then rinsed twice with distilled water.

Following the methodology suggested by Brasil (2009), with adaptations, the germination method was carried out on moistened Germitest® paper with 2.10 mL of distilled water each, equivalent to 2.5 times the weight of the dry paper.

Firstly, the best light conditions for the germination of passion fruit seeds (*P. edulis* f. *flavicarpa*) were determined using fruits obtained from manual cross-pollination. Then, the seeds were randomly selected and divided into two treatments with four replicates, with 25 seeds per replicate, totaling 100 seeds evaluated in each treatment for a total 200 seeds. The treatments were arranged in a factorial following a Completely Randomized Design (CRD): T1 – Dark / Controlled Temperature, T2 – Light / Controlled Temperature.

To ensure complete darkness for 24 hours, the plates were placed in black Gerbox® boxes and maintained in a BOD incubator at controlled temperature. The average temperature during the experiment was 23 ± 2°C, with a maximum of 25.3°C and a minimum of 20.9°C. For the treatment exposed to light, illumination was provided according to ambient conditions, totaling 11 hours and 27 minutes of light per day. Evaluations were conducted daily for 28 days, starting from the first day of incubation. Seeds were considered germinated upon radicle emergence of at least 1.0 mm and remained in Petri dishes until the end of the evaluation period.

At the end of the experiment, the following variables were analyzed: mean germination time, germination speed index, and germination rate. The data were subjected to tests for normality and homogeneity of variances, followed by analysis of variance (ANOVA). Mean com parisons were performed using Tukey's test at a 5% significance level.

Influence of floral visitors on seed germination

After the definition of the best light conditions for the germination of passion fruit seeds, the second experiment was carried out to compare the viability of seeds and the development of seedlings originating from fruits formed from different floral visitation conditions.

According to methodology suggested by Brasil (2009), with adaptations, seeds were placed on petri dishes containing Germitest paper and kept in black Gerbox® to maintain the absence of light under average temperature of 23°C in BOD, with maximum temperature of 25.3°C and minimum temperature of 19.1°C. The study consisted of three floral visitation treatments: T1 - Monitored Free Visitation (MFV); T2 - Manual Cross-Pollination (MCP); and T3 - Free Visitation Without Pollen Thieves (FVWT), in which the visitation of *A. mellifera* and *T. spinipes* were prevented. The treatments had four replicates with 25 seeds each, totaling 100 seeds evaluated in each treatment for a total of 300 seeds.

Seeds were monitored daily for 28 days using a green safety light to prevent light absorption (Lopes & Soares, 2003). The seeds were considered germinated when the radicle emission had at least 0.1 mm in length, kept on plates petri dish until the end of evaluations. After the evaluation period, the following variables were calculated:

mean germination rate and mean germination time of each treatment; germination speed index, total seedling length (mm), shoot length (mm), root length (mm), fresh mass (mg), and dry seedling mass (mg). Means were subjected to normality and homogeneity tests, and analysis of variance. Finally, means were compared using the Tukey test at 5% probability using the Sisvar statistical software.

Results

Evaluations of the germination conditions for passion fruit seeds indicated that germination began on the 6th day after the start of the experiment in all treatments, concluding on the 13th day in treatment 1 and the 15th day in treatment 2. As shown in Figure 1, it can be inferred that, for *P. edulis* germination tests, the first count and final evaluation may be effectively conducted on the 6 and 15th days, respectively, since no additional germination occurred beyond day 15. The peak germination was observed on the 8th day, with the highest number of germinated seeds recorded in both treatments. Despite this, evaluations were extended to the 28th day, in accordance with the Rules for Seed Testing (RST) (Brasil, 2009), which recommend conducting the first count on day 7 and the final count on day 28 for *P. edulis*.

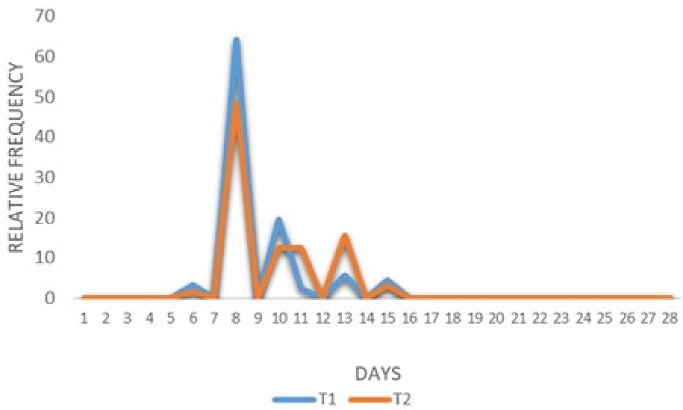

Figure 1. Relative frequency in the germination of *P. edulis* seeds over 28 days, subjected to different light conditions: T1 - Dark / Controlled Temperature, T2 - Light / Controlled Temperature.

Although germination started at the same time in both treatments, seeds in the dark (T1) showed the shortest mean germination time, demonstrating that the presence of light can interfere negatively with the germination process. As for germination speed index, treatment 1 – Dark / Controlled Temperature exhibited the highest mean seed germination speed, demonstrating that the combination of Dark + Controlled Temperature resulted in greater seed germination speed in *P. edulis* (Table 1).

Table 1. Mean germination time (days after sowing - DAS), germination percentage and germination speed index (GSI) of passion fruit seeds subjected to different light conditions, after 28 days.

Treatments	Mean Germination Time	Germination %	GSI
T1 – Dark / Controlled Temperature	10.14 a	87.00 a	0.11 a
T2 – Light / Controlled Temperature	11.43 b	64.00 b	0.10 b
CV	19.70	10.72	9.58

Means followed by the same letter in the column do not differ from each other based on the Tukey test at 5% probability.

The highest germination rates were observed in treatment with absence of light, T1, indicating that darkness is essential for good germination of *P. edulis* seeds. Further, when combined with average temperatures of 23°C, the germination response tends to be positive.

Influence of floral visitors on seed germination

Seed germination evaluations of flowers under different floral visiting conditions indicate that the first seeds began to germinate after 5 days of treatment in T2 – MCP and T3 – FVWT, revealing the highest number of seeds germinated on this day (Figure 2). The last germination occurred on the 12th day, but observations were maintained until the 28th day, as recommended by the methodology.

The shortest time, the highest germination rate and speed were observed in treatments Manual Cross-Pollination – T2 and Free Pollination Without Thieves – T3, indicating that the presence of pollen thieves in flowers (insects that take away pollen without pollinating the flowers), such as *A. mellifera* and *T. spinipes*, can interfere with the viability and seed germination rate (Table 2).

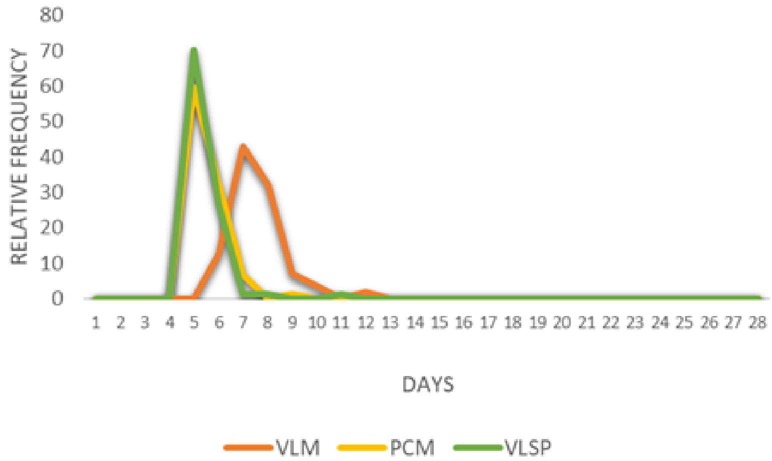

Figure 2. Relative frequency in the germination of *P. edulis* seeds over 28 days, subjected to different visitation conditions: T1 – Monitored Free Visitation (MFV), T2 - Manual Cross-Pollination (MCP) and T3 - Free Visitation Without Thieves (FVWT).

Table 2. Germination percentage and germination time (days after planting - DAS), of passion fruit seeds subjected to different conditions of floral visitation, after 28 days.

Treatments	Mean Germination Time	Germination %	GSI
T1 – MFV	7.54 b	56.00 b	0.13 b
T2 – MCP	5.50 a	88.00 a	0.18 a
T3 – FVWT	5.39 a	87.00 a	0.18 a
CV	16.64	9.49	3.15

Means followed by the same letter in the column do not differ from each other based on the Tukey test at 5% probability.

The observations lead to the assumption that the presence of insects that collect pollen but do not carry out pollination, called pollen thieves, can affect seed viability by decreasing the germination rate by 31% and, consequently, considerable loss of potential seedlings. The presence of effective pollinators, with compatible body structure, can increase seed setting. Therefore, it is believed that pollinators enable a better distribution of pollen grains and, consequently, better fertilization of ovules and ovary filling, thereby ensuring greater success in fruit setting, growth, and quality (Ariizumi et al., 2013).

Seedlings obtained from fruits subjected to Free Pollination Without Thieves – T3 exhibited the highest mean seedling length when compared to manual cross-pollination and free visitation treatments. Although the absence of light generally causes seedlings to etiolate, it is believed that the difference in average values may indicate that the presence of *A. mellifera* and *T. spinipes* in flowers results in smaller seedlings. However, shoot length was not affected, with all treatments having similar values (Table 3).

Table 3. Seedling Length – SL (mm), Aerial Part Length – APL (mm), Root Length – RL (mm), Fresh Mass – FM (mg) and Dry Mass – DM (mg) of passion fruit seeds subjected to different floral visiting conditions.

Treatments	SL (mm)	APL (mm)	RL (mm)	FM (mg)	DM (mg)
T1 – MFV	60.58 b	20.09 a	40.58 b	13.0 b	10.05 a
T2 – MCP	52.87 c	20.39 a	32.42 c	17.8 a	10.07 a
T3 – FVWT	70.51 a	24.45 a	46.23 a	19.9 a	10.55 a
CV	29.64	51.65	33.18	58.78	58.76

Means followed by the same letter in the column do not differ from each other based on the Tukey test at 5% probability.

The longest root length was also found in seedlings subjected to free pollination without thieves – T3, while manual cross-pollination (MCP) resulted in seedlings with shorter root size (Table 3).

Regarding seedling fresh mass, cross-pollination – T2 and free pollination without thieves – T3 did not differ for this parameter; however, free visitation resulted in the lowest mean seedling mass values. Considering seedling dry mass, no variation between average values was observed. Therefore, there are no differences between seedlings subjected to T1 – Monitored Free Visitation, T2 – Manual Cross-Pollination and T3 – Free Visitation Without Thieves because the dry mass parameter is the most significant parameter for seedling evaluation.

Discussion

Seed germination began on the sixth day in all treatments after the implementation of the experiment, a period similar to that reported by Zucareli et al. (2015). Such uniformity in the germination process can enhance seedling production efficiency on a commercial scale (Alexandre et al., 2004).

The highest germination percentages were observed in the treatment exposed to darkness, a pattern also reported by Zucareli et al. (2015), who found the highest average germination rates for *P. edulis* under dark conditions, regardless of temperature. Similarly, Zucareli et al. (2009) observed greater germination in *P. cincinnata* seeds maintained in constant darkness. Therefore, it can be inferred that 12 hours of light exposure negatively affects the germination of *P. edulis*, with higher germination percentage, faster germination speed, and shorter mean germination time achieved only under dark conditions.

Influence of floral visitors on seed germination

Regarding the germination of seeds from flowers subjected to different pollination conditions, Alexandre et al. (2004) reported that seedling emergence in passion fruit crops typically occurs irregularly, varying from ten days to three months, which hinders uniform seedling formation. However, in the present study, germination occurred over a shorter interval and with greater regularity, an important trait for producing uniform seedlings more efficiently.

In a similar study, Santos et al. (2015) evaluated the germination of eight *P. edulis* genotypes and observed variation consistent with the results of this study for Manual Cross-Pollination (ranging from 80.5 to 98.5%). For Free Visitation, germination rates ranged from 81 to 99.5%, likely due to a high frequency of effective pollinators and the absence of thieves in the study area, a condition that differs from the present work. In pink pepper (*Schinus terebinthifolia*) cultivation, Cruz et al. (2005) observed that manual and bee-assisted pollination resulted in a greater number of seeds compared to free pollination, demonstrating that the presence of only effective pollinators is beneficial.

The average seedling length observed in fruits subjected to free pollination without thieves (T3) was similar to that reported by Santos et al. (2015). The authors reported mean shoot lengths at 28 days ranging from 28.01 to 34.88 mm for Free Visitation seedlings and from 28.61 to 39.68 mm for those under Manual Cross-Pollination.

Root length was also greatest in seedlings from fruits exposed to Free Pollination Without Thieves (T3), suggesting that the exclusive presence of effective pollinators enhances ovule fertilization, subsequently supporting improved root development.

With respect to seedling fresh mass, higher mean values were reported by Santos et al. (2015), ranging from 18 to 30 mg under Free Visitation and from 19 to 32 mg under Manual Cross-Pollination. This difference may be attributed to the use of sand as the germination substrate in their study, which can provide nutrients. In contrast, the present study used Germitest paper, where the only nutrient source was the seed’s own reserves.

Conclusion

The constant absence of light favors the mean germination rate and time of *P. edulis* seeds. Seeds from fruits resulting from free floral visitation, with and without the presence of thieves (MFV and FVWT) germinated more in a shorter time. The absence of thieves in pollination also resulted in seedlings with greater root length and such aspects may benefit the commercial production of yellow passion fruit seedlings (greater uniformity). The presence of pollen thieves resulted in a 31% loss in germination, but there were no differences in seedling formation in MFV, MCP and FVWT, considering the uniformity of dry matter between treatments.

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Data availability

Not applicable.

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