



Influence of desiccation time and method on pollen viability, stigmatic receptivity and fruit quality of *Passiflora edulis* Sims

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Received: 23 September 2025 / Revised: 26 February 2026 / Accepted: 3 March 2026 / Published online: 16 March 2026
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Abstract

The present study was conducted to investigate the effect of desiccation time and methods on *P. edulis* pollen viability, stigma receptivity, pistil-pollen interaction and fruit quality assessment. For this, fresh pollen (control) and dried pollen grains were distributed in Petri dishes and subjected to drying in a warm-air oven or silica gel, both for periods of 20, 40, 60 and 120 min. In vitro pollen germination, histochemical evaluation, in vivo pollination, fruiting and seed formation tests showed better performance of pollen samples desiccated in silica gel for 40 min. Stigmas of *P. edulis* were highly receptive (100%) during the periods from 2:00 to 3:20 p.m. These findings can support efficient pollen management methods to increase pollination efficiency of *P. edulis* under moist environmental conditions, as well as to support breeding and conservation programs.

Keywords Pollen germination · Conservation · Breeding · Abiotic stress · Pollination · Fruit set

1 Introduction

Yellow or sour passion fruit (*Passiflora edulis* Sims) belongs to the genus *Passiflora* L, which is considered the most representative of the Passifloraceae Juss family. This genus comprises around 500 species, most of which are native to tropical and subtropical regions worldwide (Sanyal et al.

2023; Yang et al. 2025). In particular, these *Passiflora* species are adapted to various Brazilian habitats and have high commercial value due to their attractive sensory, nutritional (Fonseca et al. 2025; Zai et al. 2025), medicinal (Borges Filho et al. 2025; Suciú et al. 2025) and ornamental properties (Mikovski et al. 2019; Souza et al. 2023). Brazil is one of the world's main producers and consumers of passion fruit. Output in 2024 reached more than 736 thousand tons, grown in 47 thousand hectares, with average yield of around 15 t.ha⁻¹.year⁻¹ (IBGE 2024).

Sour passion fruit is an allogamous and self-incompatible plant, which makes self-pollination unfeasible. Therefore, in the absence of natural pollinators such as bumblebees (*Xylocopa* spp.) – the main natural pollinators of yellow passion fruit – artificial pollination is necessary (Soares et al. 2018). Artificial pollination has been extensively used by researchers and growers to enhance production for the fresh fruit trade.

The effects of abiotic factors on pollen viability have been observed for many angiosperms, with different degrees of sensitivity and responses (Patel et al. 2025). Pollination can be affected by climatic conditions, particularly air humidity. At the extremes, high and low humidity can affect pollen-pistil interaction and make pollination difficult. In particular, high humidity and temperature accelerate pollen aging,

Communicated by Jin Hoe Huh

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reduce longevity, and can cause premature pollen membrane rupture (Pacini and Dolferus 2019; Iovane et al. 2022). In the case of desiccation-sensitive pollen, low humidity can increase the risk of water loss, leading to impaired membrane integrity and reduced germination rates (Franchi et al. 2011). Therefore, the availability of viable *ex situ* conserved pollen is critical to ensure pollination efficiency during unfavorable conditions, avoiding negative impacts on productivity, especially in passion fruit production centers. Furthermore, it allows the future use for artificial crosses in breeding programs with *Passiflora* species that present asynchronous flowering.

Although studies have reported that pollen of *Passiflora* is recalcitrant in relation to desiccation tolerance (Anushma and Rajasekharan 2023), information is still scarce about cultivated species of *P. edulis*. Such knowledge can help to support studies aimed at developing pollen conservation strategies. In turn, this can impact breeding and crop management with conserved pollen for fruit production even in the presence of biotic and abiotic stresses. Pollen desiccation can be achieved using simple physical processes such as air drying, calcium sulfate application, use of hygroscopic substances such as silica gel, or more complex techniques such as lyophilization or use of chemical desiccants like amyl acetate and xylene (Quaresma et al. 2021; Guollo et al. 2022). There are some works in the literature that have evaluated pollen viability using different drying method (Gaudet et al. 2020; Impe et al. 2022; Liu et al. 2023), so far, there are no studies of this nature involving *Passiflora edulis*. The amount of water that can be removed from pollen grains varies depending on the species. In *Gleditsia sinensis* Lam., for example, the removal of water from pollen reduces its viability (Liu et al. 2023), while in *Petunia hybrida*, pollen viability was reportedly maintained (Nepi et al. 2010).

As a starting point to overcome the challenges faced by *Passiflora edulis* productivity in cultivated areas, particularly under adverse climatic conditions characterized by excessive rainfall or high humidity, it is critical to understand the consequences of desiccation on pollen viability and in vivo pollination. To our knowledge, this is the first study of *P. edulis* systematically investigating multiple controlled desiccation regimes (method $\times$ time), quantifying their effects on pollen water content and viability, and then linking those conditions to in vivo pollination success (fruit set, seed production) and post-harvest fruit quality traits. The results of this study will provide technical support for artificial pollination breeding and management for fruit production and future pollen conservation strategies.

2 Material and methods

2.1 Study site and plant material

The study was carried out at the experimental field of Embrapa Mandioca e Fruticultura located in the municipality of Cruz das Almas, Bahia, Brazil (12° 39' 25" S, 39° 07' 27" W, 226 m). According to the classification of Köppen (1928), the climate of the region is transition from Am to Aw types (tropical sub-humid to dry), with average annual air temperature of 23.8 °C, average annual precipitation of 1224 mm (concentrated from June to August), and yearly average relative humidity of around 80%. The experiment was carried out from July to August 2023. The average temperature and relative humidity during this period were 20 °C and 76% respectively (Meteorological Station of the Embrapa Mandioca e Fruticultura). The genotype used was BGP 418 (*P. edulis*), belonging to the Passion Fruit Germplasm Bank of Embrapa Mandioca e Fruticultura. Flowers (anthesis) from different *P. edulis* plants were collected in the field and immediately taken to the Tissue Culture Laboratory.

2.2 Anther water content determination

To quantify anther water content (AWC), 90 anthers from 18 *P. edulis* flowers were used. In the laboratory, these anthers were subjected to desiccation in aluminum foil containers (2 $\times$ 2 cm) using two different methods (warm-air oven desiccation at 30 $\pm$ 2 °C or silica gel desiccation) at five time intervals: 0 (control - fresh pollen), 20, 40, 60 and 120 min. For each treatment, three replicates containing three anthers each were evaluated. The anthers underwent drying in a desiccator (300 mm diameter) containing silica gel (500 g) at ambient temperature or in a warm-air oven desiccation at 30 $\pm$ 2 °C. The calculation of anther water content was performed utilizing the formula: $AWC = (\text{fresh anther weight} - \text{dry anther weight}) / \text{fresh anther weight} \times 100\%$.

2.3 In vitro germinability and pollen tube growth of fresh and desiccated pollen

To quantify in vitro pollen germination and pollen tube growth, 100 anthers were collected from different flowers at anthesis. Subsequently, the anthers were subjected to desiccation in silica gel or in a warm-air oven at different times (0 min – control, 20, 40, 60, and 120 min). For each treatment, a pool of pollen grains from ten randomly collected anthers was used. The culture medium and incubation conditions used were the same as those described by Soares et al. (2018). Total pollen grain counts and the number of germinated pollen grains were determined in ten microscopic

fields of view for each Petri dish. The experiment was a completely randomized design in a 5×2 factorial scheme (desiccation time $\times$ desiccation method), consisting of three Petri dishes and 30 replicates for each treatment.

To determine the pollen tube growth, we selected 30 tubes at random from each Petri dish, totaling 90 pollen tubes per treatment. The pollen tube length (mm) was measured with the ImageJ 1.46r software (Rasband 1997–2016) utilizing the images obtained with the stereomicroscope at 25x magnification. The experimental design was completely randomized in a 5×2 factorial scheme (desiccation time $\times$ desiccation methods) with 90 repetitions.

2.4 Evaluation of fresh and desiccated pollen viability using Alexander's solution

One hundred anthers from different flowers collected in anthesis were desiccated in silica gel or in a warm-air oven for 20, 40, 60 and 120 min. The control was fresh pollen grains collected in anthesis. For each treatment, a pool of pollen grains from ten different anthers was distributed on glass slides and stained with two drops of 2% Alexander's solution (Alexander 1980). Pollen viability was assessed by the scanning method, observing 100 pollen grains per slide with three replicates, for a total of 300 pollen grains for each treatment. The experimental design was completely randomized in a 5×2 factorial scheme (desiccation times $\times$ desiccation methods) in three replicates, where each replicate was represented by one slide.

2.5 Stigma receptivity

Before the experiment, the flower buds, ready to open, were protected and labeled. After the flowers were fully opened (anthesis – at around 2:00 p.m.), they were collected until the last pollination was completed. Five flower collection times were evaluated (2:00, 2:45, 3:23, 4:06, 4:40 p.m.). For each time, 15 flowers from different plants were sampled; as each flower contained three stigmas, resulting in 45 stigmas evaluated per treatment. The stigmas ($n=45$) were completely immersed in an alpha-naphthyl acetate solution in phosphate buffer, acetone and fast blue B salt for approximately five minutes and then washed in distilled water to detect esterase activity (Dafni 1992). Stigma receptivity was estimated by assigning degrees of receptivity, as adapted from Dafni and Maués (1998). This procedure was performed to ensure that all the field experiments were conducted in a period of high stigma receptivity.

2.6 Analysis of pollen-pistil interaction via fluorescence microscopy and in vivo pollen germination

Pollen tube growth was determined by collecting the pistils 24 h after pollination with fresh pollen (control) and with pollen dried in silica gel or in the warm-air oven for 20, 40, 60 and 120 min. Five pistils were collected for each treatment, resulting in 50 pistils analyzed. The pistils were fixed and stained, according to the methodology described by Soares et al. (2018). The number of pollen grains attached to the stigma, pollen germination and the development of the pollen tube through the style and ovary were recorded (Reger and Sprague 1982). Pollen germination percentage on the stigma surface was calculated as the proportion of pollen grains germinated to the total number of pollen grains adhered, on a scale of 100. The percentage of fertilized ovules was calculated from the proportion of ovules penetrated by pollen tubes in relation to the total number of ovules observed $\times 100$. For each treatment, five microscopic slides were prepared, containing one pistil for each slide. Pollen tubes observed penetrating the ovarian micropyle were presumed to have caused fertilization, as suggested by Chaix and Marchais (1996). The experimental design was completely randomized in a 5×2 factorial scheme (desiccation times $\times$ desiccation methods) in five replicates, where each replicate was represented by one microscopic slide.

2.7 Effect of pollen desiccation on seed and fruit production

To understand the effect of desiccation time and method on seed and fruit production, cross-pollination of 150 flowers in anthesis was carried out. One day before the pollination, the flowers were covered with fine-mesh nylon bags before anthesis to guarantee there was no contamination by pollinating agents. For each desiccation time (0 min – control, 20, 40, 60, and 120 min) and desiccation method (silica gel or warm-air oven) 30 emasculated flowers were manually pollinated with pollen pool collected from 50 flowers at anthesis and from ten different plants. After this, the flowers were identified and covered until fruit harvest. The percentage of fruiting was evaluated 30 days after pollination by counting the fruit formed in relation to the number of pollinated flowers. After fruit ripening, the number of seeds per fruit was recorded.

2.8 Physical-chemical characteristics of fruits formed after pollination with desiccated pollen

The fruits resulting from crosses using pollen dried in the warm-air oven or with silica gel at the different times were

harvested for analysis when more than 50% of their peel showed yellow coloration. After harvesting the fruits, we evaluated the following physical-chemical traits: fruit weight (FRW), peel weight (PEW), pulp weight with seeds (PUWs) and juice yield (JY) (Jesus et al. 2017). The first three variables were measured in grams, while the juice yield was measured in percentage. The physical-chemical evaluation was carried out with a total of 50 fruits (5 fruits for each treatment). The experimental design was completely randomized in a 5×2 factorial scheme (desiccation times $\times$ desiccation methods) with five replicates per treatment.

2.9 Statistical analysis

The percentage data were transformed into arcsine square root for standardization and homogeneity of variances. All data were subjected to analysis of variance (ANOVA). Means were compared by the t-test (desiccation method) and by the Scott-Knott test (desiccation time and stigma receptivity) at 5% probability. For the in vivo pollen germination, fertilized ovules, fruit weight, peel weight, pulp weight with seeds and juice yield, we employed boxplots for descriptive analysis using the “ggplot2” package implemented in the R software. Data are shown as mean $\pm$ standard error, except for stigma receptivity. All analyses were performed using the R statistical software, version 4.0.3 (R Development Core Team, 2022).

3 Results

3.1 Anther water content

We observed a highly significant effect ($p \leq 0.001$) of the factors (desiccation times and methods) regarding the anther water content (AWC), as reported in Table 1. The interaction between them had no significant effect ($p \geq 0.05$). During the experiment, the initial water content of fresh pollen was 65.3% at 105 °C in the warm-air oven and 57.9% with

silica gel desiccation (Table 1). A reduction in anther water content was recorded with increasing desiccation period according to both desiccation methods tested. The 120 min period provided the greatest dehydration of the pollen grains, with reductions in water content of 15.3% and 15.7% when desiccated in the warm-air oven and silica gel, respectively (Table 1). Regarding the desiccation methods tested, similar behavior was observed in the reduction of the anther water content. The dehydration with silica gel provided the following anther water contents: 54.6% (20 min), 51.6% (40 min), 48.1% (60 min) and 42.2% (120 min) (Table 1). On the other hand, warm-air oven desiccation reduced the water contents to 61% after 20 min, 57.8% after 40 min, 55.5% after 60 min and 50.0% after 120 min (Table 1).

3.2 In vitro pollen germination and pollen tube growth

According to the in vitro pollen germination, there were highly significant differences ($p \leq 0.001$) between desiccation times, methods and their interaction. Pollen germination significantly varied among the methods and different desiccation times used (Figs. 1A and 2A–J). Fresh pollen (control) achieved average in vitro pollen germination percentages of 54.7%. Pollen grains dried with silica gel for 40 min achieved a significantly ($p \leq 0.001$) higher germination percentage with 83.7% (Fig. 1A). The warm-air oven treatments also showed significantly ($p \leq 0.001$) higher germination percentage at 40 min (64.6%) (Fig. 1A). A reduction in germination values was observed with further decreases in water content, beginning to decrease after 60 min of desiccation, reaching low values. such as 16.8% when the pollen was dried for 120 min in silica gel and 7.0% in warm-air oven (Fig. 1A), indicating that the pollen grains did not tolerate further desiccation. The pollen tube growth also indicated a similar trend; treatment with silica gel desiccation for 40 min was significantly superior ($p \leq 0.001$), reaching the maximum development with pollen grains dried in the warm-air oven (1.8 mm) and silica gel desiccation (2.68 mm) (Fig. 1B). On the other hand, lower levels of this variable were observed when the pollen grains were subjected to desiccation for 120 min, with 0.38 mm (warm-air oven) and 0.42 mm (silica gel desiccation).

3.3 Histochemical analysis of pollen grains

Based on the pollen viability results of staining with Alexander’s solution, there were highly significant differences ($p < 0.0084$) between desiccation times, desiccation methods and their interaction (Fig. 1C). Staining of pollen grains with Alexander’s solution allowed distinguishing viable pollen grains – stained pink (Fig. 2K – full arrow) from

Table 1 Anther water content and water loss measured immediately after different desiccation times

Desiccation time (minutes)	Water content (%)		Water loss (%)	
	Warm-air oven	Silica gel desiccation	Warm-air oven	Silica gel desiccation
	65.3 $\pm$ 0.79 a	57.9 $\pm$ 1.56 a	0	0
20	61.0 $\pm$ 0.23 b	54.6 $\pm$ 0.20 b	4.2 $\pm$ 0.24	3.2 $\pm$ 0.20
40	57.8 $\pm$ 0.51 c	51.6 $\pm$ 0.13 c	7.5 $\pm$ 0.50	6.2 $\pm$ 0.13
60	55.5 $\pm$ 1.01 d	48.1 $\pm$ 0.41 d	9.8 $\pm$ 1.00	9.8 $\pm$ 0.41
120	50.0 $\pm$ 0.68 e	42.2 $\pm$ 3.70 d	15.3 $\pm$ 0.62	15.7 $\pm$ 3.69

Values represent the mean $\pm$ SD (standard deviation) of quintuplicate detection. Lowercase letters in the same column indicate significant differences in anther water content among different desiccation times by the Scott-Knott grouping test ($p \leq 0.05$)

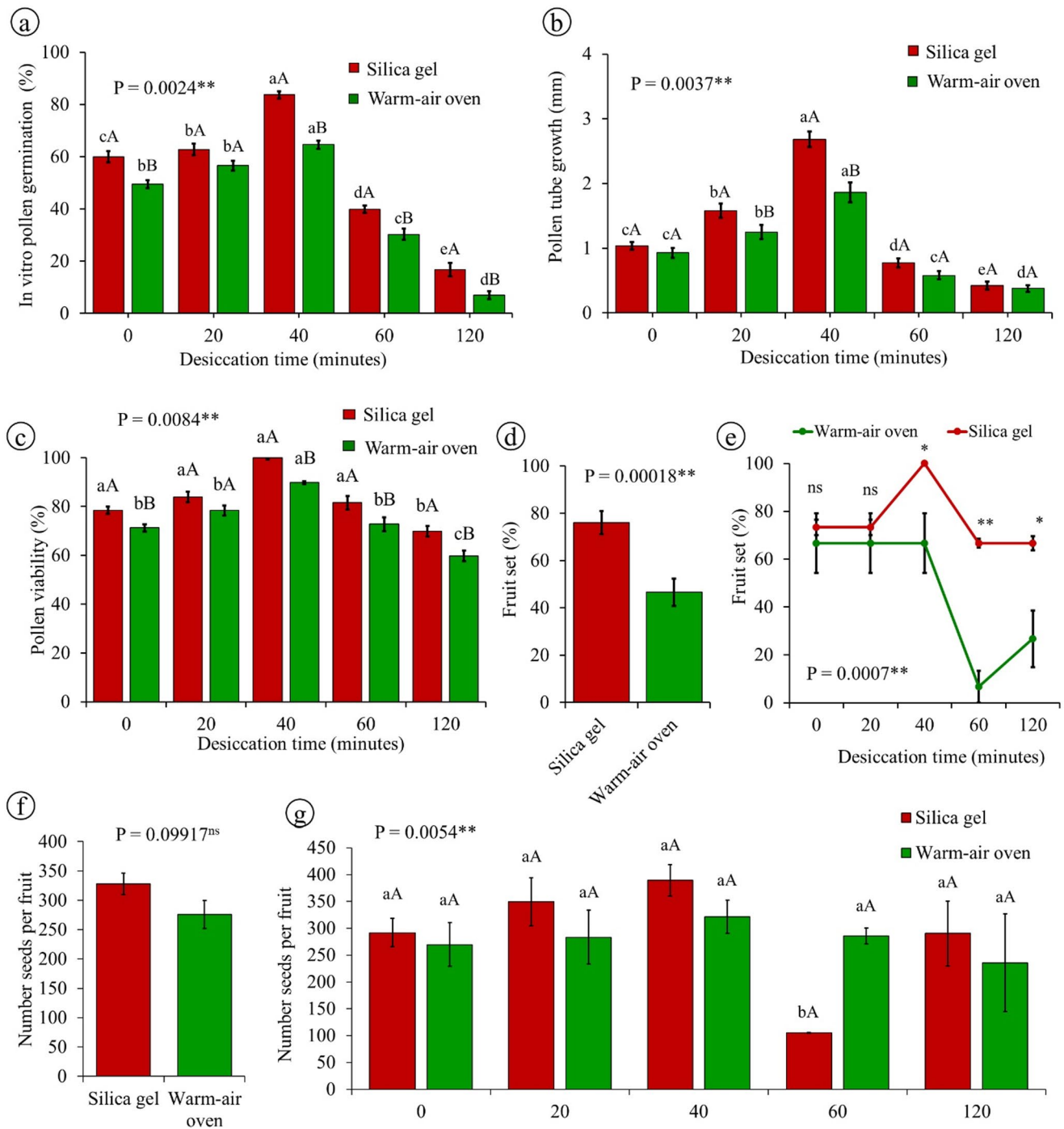


Fig. 1 The interaction effect between desiccation method (warm-air oven and silica gel) and different times on pollen viability of *P. edulis*. **(A)** In vitro pollen germination. **(B)** Pollen tube growth. **(C)** Viability according to Alexander's solution. **(D-E)** Fruit set. **(F-G)** Number of seeds per fruit. Means followed by different lowercase letters indicate statistically significant differences among desiccation times within each desiccation method (bars of the same color refer to each des-

iccation method within different desiccation times), according to the Scott-Knott test ($p \leq 0.05$). Means followed by different uppercase letters indicate significant differences between desiccation methods within each time (bars of different colors refer to different desiccation methods within each desiccation time), according to Tukey's test ($p \leq 0.05$). ns=not significant, * $p \leq 0.05$, ** $p \leq 0.01$, respectively, by the F-test. Data are expressed as mean $\pm$ standard error (bars)

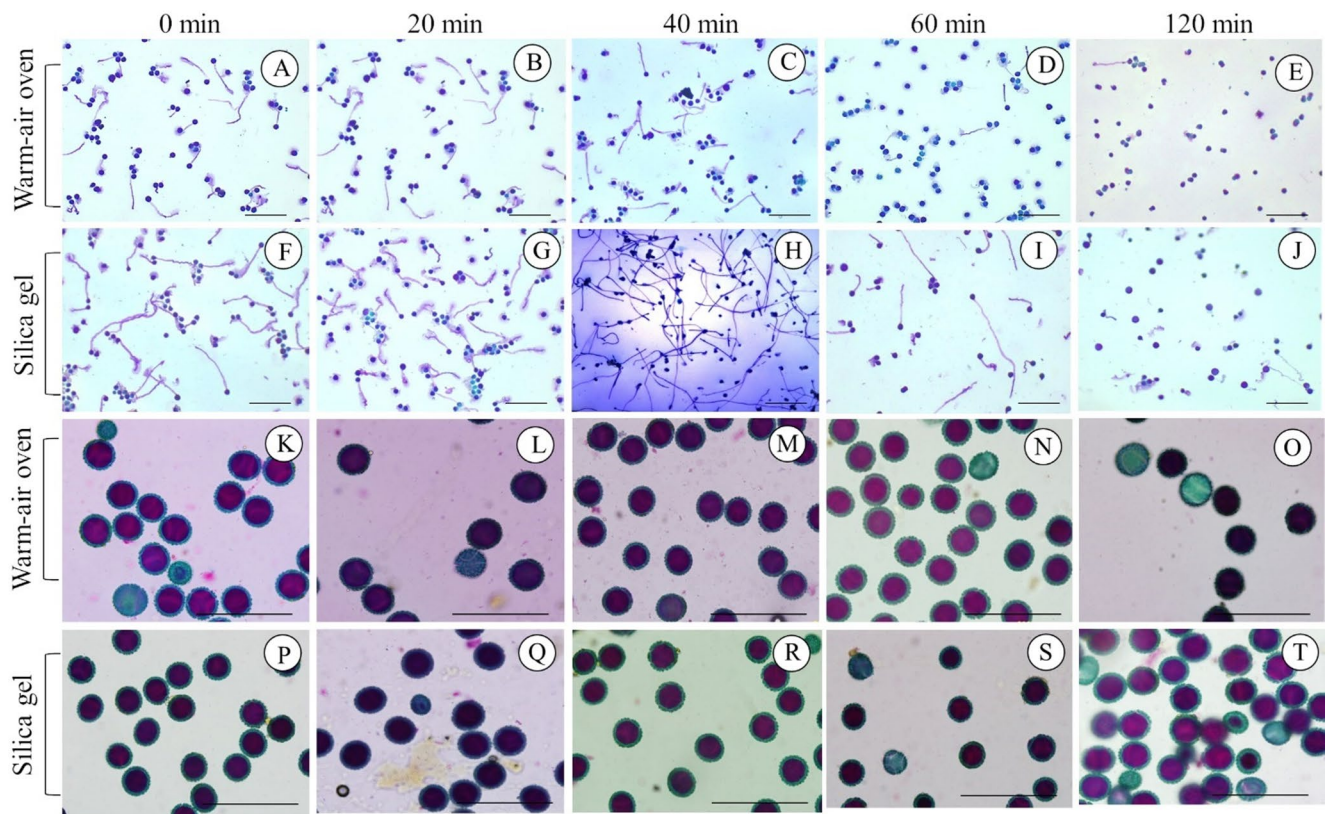


Fig. 2 In vitro pollen germination (A–J) and pollen viability based on Alexander's solution (K–T) after submission to different desiccation times (0 to 120 min), and desiccation methods (warm-air oven and silica gel). Scale Bar: 100 μ m (A–J), 200 μ m (K–T)

nonviable grains – stained blue (Fig. 2K – dotted arrow). Fresh pollen grains showed viability ranging from 71.32% to 78.5% (Fig. 1C).

Between the pollen desiccation methods, generally pollen grains dried in silica gel showed a higher proportion of stained pollen grains, with variation of 83.9% to 99.9% when compared to the warm-air oven method, with 59.8% to 80.8% (Fig. 1C). Regarding desiccation time, pollen dried for 40 min achieved the highest percentage of viable pollen grains, with values above 80%, regardless of the drying method tested. After this period (40 min.), pollen viability decreased to 59.8% under warm-air oven conditions and to 69.9% in silica gel desiccation, when the pollen was subjected to drying for 120 min (Fig. 1C). For the time and desiccation method interaction, pollen grains desiccated in silica gel for 40 min had the greatest viability, of 99.9%.

3.4 Stigma receptivity

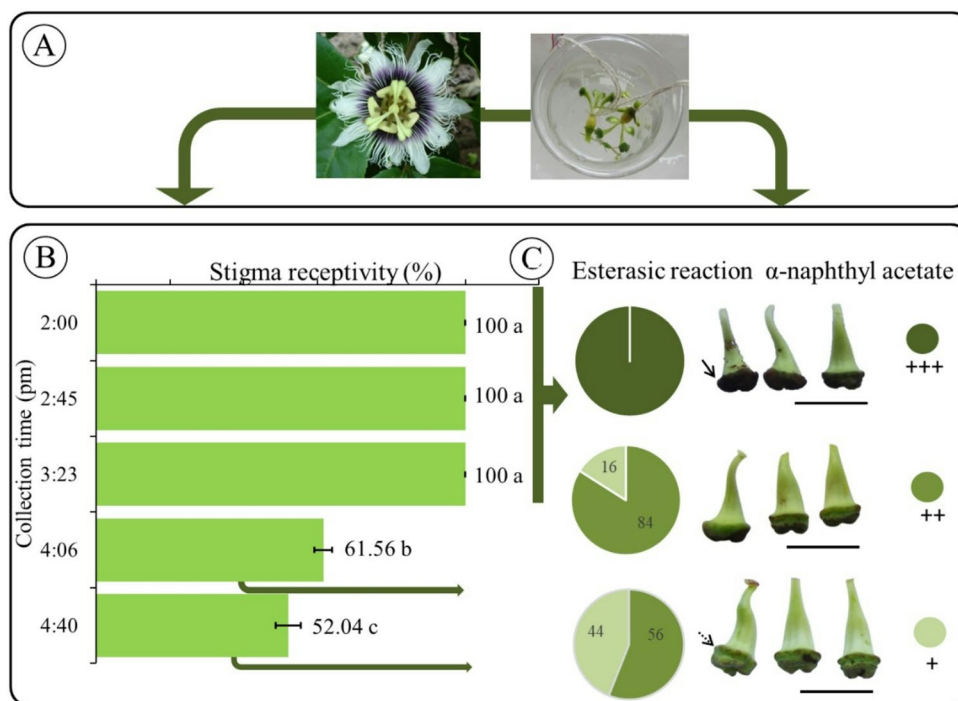
The period during which the stigmas were collected significantly influenced ($p < 0.001$) their receptivity (Fig. 3A). The peak receptivity occurred between 2:00 and 3:00 p.m., with high esterase activity (100%) (Fig. 3B). Esterase activity is identified by the dark brown color (Fig. 3C – full arrow), mainly in the region of the stigmatic papillae, possibly

related to the presence of exudates that commonly occur in this region. However, stigma receptivity progressively decreased starting 2 h after anthesis, specifically between 4:06 (84%) and 4:40 p.m. (56%), as identified by the light brown coloration (Fig. 3B, C – dotted arrow).

3.5 In vivo pollination, fruit set and seed production

Hand pollination of flowers (artificial pollination) with pollen dried in silica gel and warm-air oven subjected to different drying times exhibited significant differences ($p \leq 0.05$) in fruit set percentage (Fig. 1D–E). In general, the highest fruit set values were obtained using pollen samples dried in silica gel (Fig. 1D). The highest fruit production was observed for pollen dried with silica gel for 40 min, achieving 100% fruit production (Fig. 1E). From this desiccation period onward, there was a reduction in fruit production, and the lowest levels came from crosses with pollen dried in the warm-air oven for 60 min (6.7%) and 120 min (26.7%). This decreasing tendency was the same as that observed in crosses carried out with warm-air oven-dried pollen. Regarding number seed per fruit, the highest values (390 seeds per fruit) were obtained from fruits obtained from pollination with pollen dried in silica gel for

Fig. 3 Stigma receptivity of *Passiflora edulis*. Stigmas collected from fully open flowers and immersed in alpha-naphthyl acetate solution (A). Receptivity percentage of stigmas collected at different times after anthesis (B). Esterase reaction: (+) weak positive reaction; (++) strong positive reaction; (+++) very strong positive reaction (C). Means followed by different letters are significantly different according to the Scott–Knott test ($p \leq 0.01$). Data are expressed as mean $\pm$ standard error (bars). Scale Bar: 1.5 cm



40 min ($p=0.09917$). However, there was no difference of seed production obtained from fruits pollinated with pollen dried in the warm-air oven for the same desiccation time (Fig. 1F–G).

3.6 Pollen-pistil interaction

Fluorescence microscopy (FM) with aniline blue staining allowed us to clearly visualize the pollen-pistil interaction of *P. edulis* (Fig. 4). In general, after 24 h of artificial pollination, both fresh (Fig. 4A, K) and dried pollen grains germinated (Fig. 4B–E; L–O) on the stigma surface. The pollen tubes grew into the stylar tissue (Fig. 4C) and ovary, reached the ovules and entered through the micropyles into the embryo sac (Fig. 4F). Furthermore, the presence of tectorial trichomes distributed throughout the stylar tissue and also in the ovary was observed (Fig. 4A).

Further in relation to the pollen-pistil interaction, there was a significant effect ($p \leq 0.05$) between desiccation methods and times, as well as the interaction between them (Fig. 5). In the case of pistils pollinated with fresh pollen (without desiccation), 69.3% of the pollen grains adhered to the stigma surface germinated in vivo (Fig. 5A), and only 38% of the pollen tubes were observed inside the ovary (Fig. 5B). In contrast, the pistils pollinated with pollen dried for 40 min in silica gel (AWC=51.6%) produced a higher proportion of pollen germinated on the stigmatic surface (90.1%) (Fig. 5A) and also a higher percentage of pollen tubes that reached the ovary (87.9%) (Figs. 4R and 5B). In the case of the pistils resulting from pollination with

warm-air oven desiccation for 40 min, many germinated pollen grains were observed on the stigma surface, with 82.1% (Figs. 4C and 5A), while only 68.4% of the pollen tubes were able to enter the ovary (Figs. 4H and 5B).

Fluorescence microscopy showed a gradual decrease in pollen germination on the stigmatic surface, with 54.3% and 28.2%, from the pistils pollinated with pollen dried in silica gel for 60 min (AWC=48.1%) and 120 min (AWC=42.2%), respectively (Fig. 5A). This trend was also observed for the penetration of pollen tubes into the ovary, since under these conditions there were reductions in this variable to values of 23.8% (60 min) and 10.3% (120 min). Additionally, pistils pollinated with pollen dried in silica gel for 120 min had some pollen deformities, such as disruption of the exine of many pollen grains (Fig. 4O) and low pollen tube activity near the ovules (Fig. 4T). Similar behavior was observed for pollen dried in the warm-air oven, which also showed a progressive decline in both pollen germination on the stigma 43.6% at 60 min; 15.5% at 120 min (Fig. 5A) and pollen-tube penetration into the ovary (19.6% at 60 min; 5.2% at 120 min) (Fig. 5B).

3.7 Physical-chemical characteristics of fruits formed after pollen desiccation

The results of the physical and chemical characteristics of fruits, namely fruit weight, peel weight and pulp weight, resulting from controlled pollination with desiccated pollen can be seen in Fig. 6. There were no significant differences ($p \geq 0.05$) in relation to the methods and desiccation times

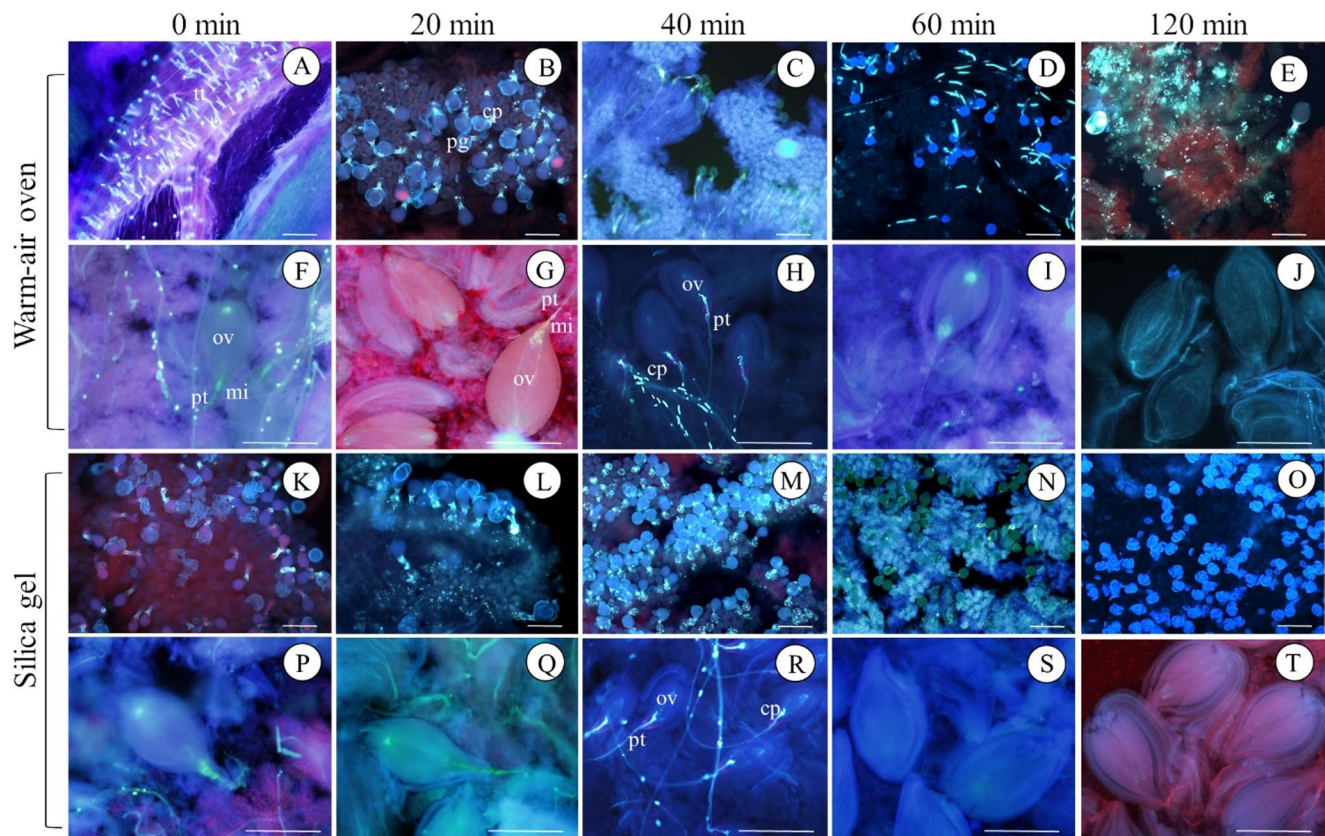


Fig. 4 Photomicrographs using fluorescence microscopy of pollen-stigma interaction of *P. edulis* after desiccation for different times. Presence of tectorial trichomes (A). High in vivo germination percentages on the stigma surface with the increase of desiccation time (A–C) in warm-air oven and in silica gel desiccation (K–M), with emphasis on 40 min in silica desiccation (M). A reduction in pollen germination on the stigma surface can be observed after pollen desiccation for 60 and 120 min (warm-air oven desiccation: D–E; silica gel desiccation:

N–O). Pollen tubes from fresh pollen and grains dried up to 40 min penetrating the micropyle and reaching the embryo sac, with consequent fertilization (warm-air oven desiccation: F–H; silica gel desiccation: P–R). Prolonged desiccation (over 40 min) considerably reduced the number of fertilized ovules (warm-air oven desiccation: I–J; silica gel desiccation: S–T). Scale Bar: 200 µm. pg= pollen grains; pt= pollen tube; cp= callose plug; ov= ovule; mi= micropyle, tt= tectorial trichomes

tested. The same applies to the interaction between factors (methods x drying times).

4 Discussion

To the best of our knowledge, there are no reports in the literature demonstrating that *P. edulis* pollen deposited on stigma with high water content, whether by floral visitors or through artificial pollination, results in low fruit-set efficiency due to reduced viability. Our findings provide new evidence of the effects of anther water content on pollen viability, and consequently of the success of manual pollination for production of yellow passion fruit (BGP418) under field conditions. However, understanding cultivar-specific responses to desiccation is essential for minimizing abiotic stress during pollination.

We observed substantial variations in the fertility of *P. edulis* (BGP418) pollen grains in relation to the time and

method of desiccation. Our results indicated a fresh anther water content of 65.3% of *P. edulis*. Some studies have reported the water content of fresh pollen in wild *Passiflora* species, with values ranging from 44.7% in *Passiflora edmundoi* Sacco to 76.1% in *Passiflora gibertii* N. E. Br. (Ferreira et al. 2024), and 46.3% in *Passiflora caerulea* L. (Anushma and Rajasekharan 2023). Notably, all of these values exceed 30%, the threshold commonly associated with recalcitrant or desiccation-sensitive pollen.

Previous studies have demonstrated that pollen grains with high water content (>30.0%), large diameter (15 to 150 µm) and spherical shape (Franchi et al. 2002) can be classified as partially hydrated or recalcitrant. In this type of pollen, desiccation can cause rapid loss of viability, with species dependent levels (Pacini and Franchi 2020; Franchi et al. 2011). In the present study, we found that water losses above 9.8% already began to cause considerable loss of pollen viability of *P. edulis*. Based on this information from the literature and the results obtained in the present study,

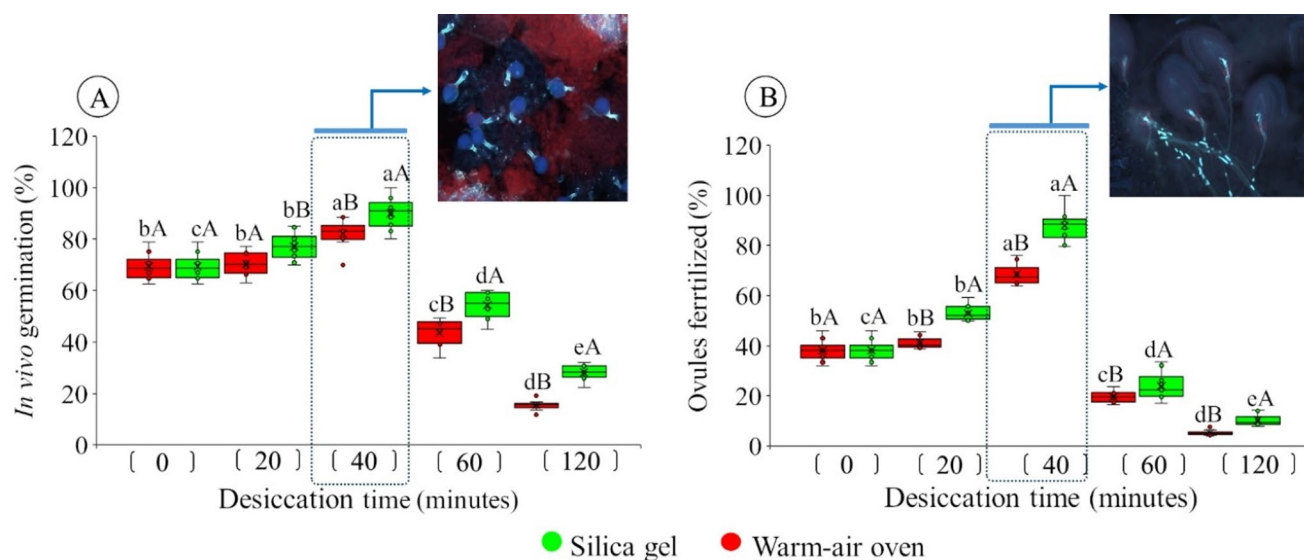


Fig. 5 Boxplots of in vivo pollen germination on the stigma surface (A) and fertilized ovules identified by fluorescence microscopy of *P. edulis* resulting from artificial pollination with warm-air oven desiccation and silica gel desiccation pollen after different drying times. The line in each box corresponds to the median and the “x” to the mean value. The different letters above the error bars indicate sig-

nificant variation between the desiccation times within each desiccation method (lowercase letters) and desiccation methods within each time (uppercase letters) according to the Scott-Knott test and F-test ($p \leq 0.05$), respectively. The circles (●) denote outliers. The whiskers (vertical line segments in each box) represent the minimum and maximum values, respectively

we can infer that the pollen of *P. edulis* can be classified as recalcitrant in relation to desiccation tolerance.

In general, a significant reduction in the anther water content (AWC) of *P. edulis* was observed as the drying time increased. This trend was common to both desiccation methods tested. Pollen dried in silica gel for 120 min showed a greater reduction in water content, although it did not differ significantly from drying after 60 min. These results are in line with those obtained by Ferreira et al. (2024), who also demonstrated that both silica gel and warm-air oven desiccation methods reduced pollen water content in eight wild *Passiflora* species. Those authors observed that longer desiccation periods further decreased AWC and pollen viability of most of the *Passiflora* species evaluated. We observed similar behavior.

In vitro germination and tube growth results enable direct analysis of several aspects of pollen performance (germination rate, tube growth rate, pollen tube integrity). In the present study, we noted significant differences for in vitro pollen germination of *P. edulis* depending on desiccation methods and times. Pollen dried in silica gel for 40 min had the highest in vitro germination percentage and tube growth, surpassing all other treatments, including those with shorter or no drying time (control), while drying times longer than 60 min resulted in more drastic reductions. These results are aligned with those reported by other authors who also observed a reduction of the in vitro germination of the *Luffa* genus after pollen desiccation for periods exceeding 40 min (Nagar et al. 2023). Additionally, silica gel desiccation of

Gleditsia silensis Lam pollen for longer periods (3–9 h) caused a significant reduction of in vitro pollen germination with the reduction of water content (Liu et al. 2023). The pollen pre-treatment time applied in this study had a determining role in the efficiency of germination in vitro and in the pollen tube growth. Previous observations by other authors support this narrative, since a major reduction in pollen water content greatly reduces its respiration and consequently its viability (Nepi et al. 2001; Wang et al. 2015).

In comparison with the assessment of in vitro pollen germination, the histochemical test is considered faster and easier for pollen viability evaluation. However, the results are usually only rough estimates of the pollen's potential to germinate, which is generally higher than the results obtained in vitro (Nagar et al. 2023; Silva et al. 2025; Raja et al. 2025; Dutta et al. 2025). In the present study, the fresh pollen of *P. edulis* presented viability above 70%. However, after drying with silica gel for 40 min was observed based on the greater proportion of colored grains, with almost 100% viability. In general, the majority of the treatments with Alexander's solution indicated greater pollen viability of *P. edulis* compared to that obtained by in vitro testing. Therefore, this test should be used to complement in vitro analysis.

Considering that yellow passion fruit is a protandrous species (Das et al. 2013; Anushma and Rajasekharan 2023) together with the results obtained here demonstrated that the stigma receptivity had a positive reaction to esterase (100%) in the first three periods tested (2:00, 2:45 and 3:23 p.m.), it

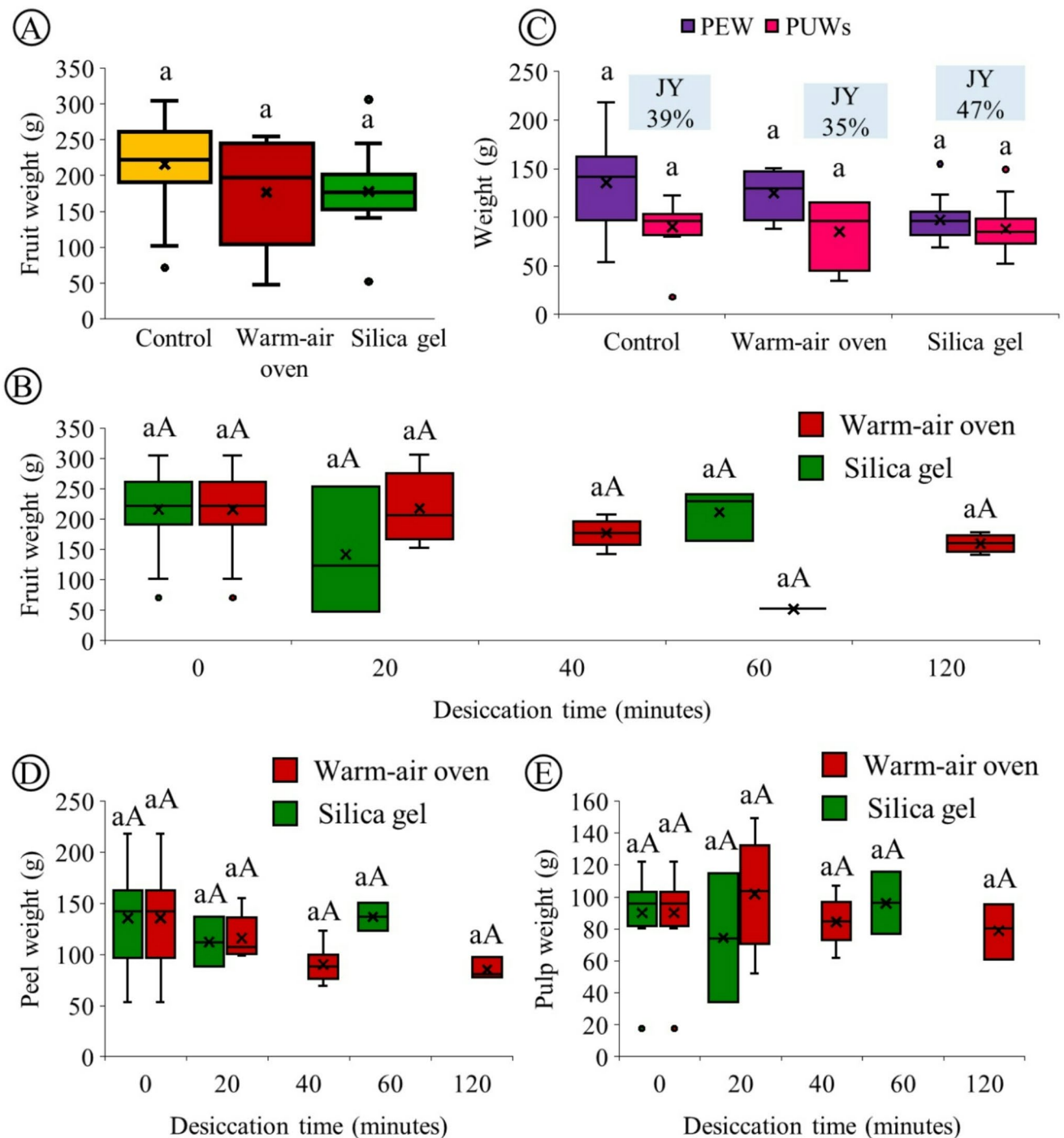


Fig. 6 Physical and chemical characterization of *P. edulis* fruits. Fruit weight obtained by different desiccation methods (A) and by interaction (desiccation method x desiccation time) (B), Peel weight (PEW), Pulp weight with seeds (PUWs) and juice yield (JY%) obtained by different desiccation methods (C). Peel weight (D), Pulp weight (E) influenced by the interaction (desiccation method x desiccation time). Means followed by different lowercase letters indicate statistically significant differences among desiccation times within each desicca-

tion method (bars of the same color refer to each desiccation method with different desiccation times), according to the Scott-Knott test ($p \leq 0.05$). Means followed by different uppercase letters indicate significant differences between desiccation methods within each time interval (different colors refer to different desiccation methods within each desiccation time), according to Tukey's test ($p \leq 0.05$) (B, D, E). Data are expressed as means $\pm$ standard error (bars). The circles (●) denote outliers

would be interesting to carry out pollination in these periods in order to maximize the efficiency of the process and ensure greater production of viable seeds. These results corroborate other studies that have reported greater esterase activity in *Passiflora edulis* (Soares et al. 2018), *Tillandsia* (Souza et al. 2020), and *Hohenbergia*. (Silva et al. 2025) at anthesis with alpha naphthyl acetate.

The pollination rate is an important criterion to measure the reproduction of passion fruit (Pan et al. 2025). In the present study, the desiccation methods and times had a significant influence on fruit set and number seed per fruit of passion fruit under field conditions. Overall, pollen grains with 6.2% water loss in silica gel desiccation resulted in 100% fruit set. The fruit set rates were higher than those observed with fresh pollen (77%). Other authors also found higher fruit set using desiccated pollen (França et al. 2010). For the variable number of seeds per fruit, a trend similar to that found for fruit set was observed, with pollen undergoing 3.2% and 6.2% water loss in silica gel desiccation producing higher seed numbers, with values of 388 and 410 seeds per fruit, respectively, although there were no significant differences between them. According to Soares et al. (2018), passion fruits from cross-pollination with fresh pollen produce an average of 406 seeds per fruit. We found greater averages when the pollen was desiccated for up to 40 min. Regarding the physical and chemical characteristics of the fruits evaluated in the present study, we found that the pollen desiccation process did not interfere in the response of the variables.

Our results may also fill the information gap on the low fruit set rate of passion fruit (BGP418) during the rainy season. In addition to providing more detailed analyses of the performance of pollen and stigma during the environmental conditions, the results obtained here may provide novel and valuable insights concerning the reproductive biology of *P. edulis* that are essential for better planning of hybridization programs and also to support future strategies for pollen conservation of this species.

5 Conclusion

Our results indicate that the pollen viability estimates of this species are influenced by desiccation methods and timing. We observed that *P. edulis* (BGP418) pollen increased potential fertility when subjected to desiccation with silica gel for 40 min by in vitro and in vivo germination tests, in both cases with findings above 80%. In this condition, the loss of water content was 6.2%, considered more effective for pollination of *P. edulis*, and can be applied in subsequent experiments. Farmers who grow passion fruit in humid places with excessive rainfall and under conditions similar

to this study can particularly benefit from this information in order to increase the efficiency of artificial pollination and consequently the production of yellow passion fruit. Further studies exploring a greater number of *Passiflora* genotypes and environmental conditions will be necessary to confirm the universality of ideal desiccation. In addition, it will be essential to determine the appropriate conditions for long-term pollen storage while preserving its viability for future use in hand pollination during flowering.

Acknowledgements The authors thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support (402359/2023-2), the research productivity fellowship (311495/2021-4) given to the third author (O.N.J.) and a postdoctoral scholarship to the second author (T.L.S. – PDS102715/2024-6). The Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the doctoral scholarship of the fourth author (I.S.S.) and Fundação de Amparo à Pesquisa do Estado da Bahia (Process: 5011/2023). The Article Publishing Charges (APC) for this research were funded by the CAPES. For Open Access purposes, the authors assigned the Creative Commons license (CC-BY).

Author contributions FFA: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft and Writing - review & editing. TLS: Conceptualization, Visualization, Investigation, Methodology, Data curation, Formal analysis, Supervision, Writing - original draft and Writing - review & editing. ONJ: Conceptualization, Visualization, Funding acquisition, Formal Analysis, Funding acquisition, Supervision, Writing - original draft and Writing - review & editing. ISS: Methodology, Investigation, Data curation, Writing-Original draft preparation. LKSL: Visualization, Investigation, Methodology, Formal analysis, Data curation, Supervision, Writing - original draft and Writing - review & editing. All authors read and approved the manuscript.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614). Conselho Nacional de Desenvolvimento Científico e Tecnológico, (PQ – 1D 311495/2021-4; 402359/2023-2 - Onildo Nunes de Jesus), (PDS 102715/2024-6 - Taliane Leila Soares)

Data availability The datasets generated or analyzed in the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest There is no conflict of interest among the authors.

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