



Floral Biology and Pollen Viability of *Passiflora edulis* Sims

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ISSO, SBCS, JNRA, JSH, PMAC, JHCSS, CALS, CEAL designed the study, wrote the protocol and wrote the first draft of the manuscript. Author VCS managed the analyses of the study. Author TSM performed the statistical analysis. All Authors managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Yellow passion fruit is a native tropical fruit tree whose cultivation has evolved very rapidly in Brazil; therefore, studies on the ecology of reproduction are needed. The study of pollen viability is an important tool to observe the male potential of the species. The objective of this study was to estimate pollen viability, pollen/ovule ratio and floral biology of *Passiflora edulis* Sims in organic cultivation located in Bananeiras, PB, and Brazilian. The study was developed in a completely randomized design, using ten flower buds in the pre-anthesis. The analyses performed in the biology laboratory of the Center of Human, Social and Agrarian Sciences of the Federal University

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of Paraíba. For the pollen viability analysis, all the anthers of the ten flower buds were used, five anthers per flower, which were crushed on a glass slide and with the aid of Alexander dye, the purple pollen grains were considered viable and the green ones unviable. To measure flower morphology and biometrics, a digital pachymeter, analytical balance and ruler were used. Five stamens form the androecium; the fillets are of short length inserted below the ovary. The flowers have three stigmas with an average height of 16.5mm, an average of 32,136 viable pollens, 58 unviable pollen grains and 140 ovules per ovary, having a pollen viability of 99.8% and a pollen/ovule ratio of approximately 95/1 pollen grains per ovule. Their structures are uniform, with no biometric variations in the amount of petals, sepals, anthers and stigma length. Alexander's reactive dye was effective in staining the pollen grains of yellow passion fruit. The pollen/ovule ratio indicates that this species performs facultative autogamy as well; however, this classification alone is not sufficient to affirm its reproductive system.

Keywords: Ecology; Passifloraceae; reproduction; yellow passion fruit; colorimetric method.

1. INTRODUCTION

The passion fruit (*Passiflora edulis* Sims) belongs to the Passifloraceae family, genus *Passiflora* [1]. Yellow passion fruit is a native tropical fruit tree whose cultivation has evolved very rapidly in Brazil [2]. According Zeraik et al. [3] this passion fruit is the most cultivated and marketed in the country due to the quality of its fruits. For [4] the growing demand for this fruit intended, for both the market-processed juice, market and the fresh fruit market.

Given its economic growth, studies on ecology of reproduction, floral biology, pollination syndrome, among others, are important. In addition, the study of pollen viability is an important tool to observe the male potential of the species. [5] Highlights that studies linked to the maintenance of pollen viability and fertility over a long period is important for the improvement and conservation of plant genetic resources.

The estimation of pollen viability is important because it allows the identification of possible infertility problems in the male gamete, where the pollen viability it closely related with normality during microsporogenesis [6].

According to Pagliarini et al. [7], there are different ways of assessing the viability of pollen grains, such as analyzing their germination potential and their ability to form seeds. However, when a faster evaluation is desired, specific chemical dyes that react with cellular components present in pollen grains sed.

The colorimetric evaluation method by Alexander's solution test based on the triple staining methodology with Orange G (intensifier), Acid Fuchsin (cytoplasm is stained red) and

Malachite green (the pollen grain wall is stained green). Thus, the nucleus of the viable pollen grain reacts with fuchsin and the fuchsin stains pink while the unviable pollen grain turns green [8].

Several studies developed about the colorimetric method to verify the pollen viability of passion fruit and attest the effectiveness of the method. According to Moreno et al. [9] the yellow passion fruit showed 88.11% of viability for the species. The use of a dye is another tool for determining the pollen viability of a species, which can highlight the male potential of a flower, and thus, subsidize the study of the ecology of certain species.

The objective of this research was to estimate pollen viability, pollen/ovule ratio and to analyze the floral biology of *P. edulis* Sims in an organic crop located in Bananeiras, PB, and Brazilian.

2. MATERIALS AND METHODS

2.1 Location of the Experiment

The analysis of pollen grains and ovules count, pollen viability performed in the biology laboratory of the Center of Human, Social and Agrarian Sciences of the Federal University of Paraíba.

Passion flower buds (*Passiflora edulis* Sims) collected in a cultivation of the experimental species installed on the first chã of the Center of Human, Social and Agrarian Sciences of the Federal University of Paraíba, located in Bananeiras. The so-called first chã is an area for growing crops of agricultural and medicinal interest.



Fig. 1. Floral bud of *Passiflora edulis* Sims

Ten flower buds were collected in March 2019 in the pre-anthesis period (before opening) at around 11am, one flower bud per plant, and then stored in 70% alcohol.

2.2 Floral Biology

To measure the morphology and floral biometry were used digital pachymeter, analytical balance and ruler. The number of petals and sepals, ovary length and diameter, number of anthers, stigma and fimbria, filament length and stigma, represented the dimensions of the floral elements. The evaluations were performed on 10 yellow passion fruit flowers (*Passiflora edulis* Sims).

2.3 Pollen Viability and Pollen Grain Count

For the pollen viability analyzes, all anthers of 10 flower buds were used, five anthers per button, totaling fifty anthers, which were crushed in a glass slide, in which, with the aid of Alexander dye [10], the purple pollen grains were considered viable, the green grains considered unviable and it was possible to verify the pollen viability. The slides were squared and spaced in millimeters to facilitate counting.

The slides were observed under BEL®photonics model 0928896 optical microscope, with a lens approaching 40x10, stained pollen grains are

considered viable, to facilitate counting were used STARFER® model 03579409 manual numerical counters.

The average pollen amount per anther and flower was determined and the viability was represented as a percentage, in which the amount of viable and unviable pollen was divided by the total pollen amount and multiplied by 100 following the equation (Eqn. 1) proposed by Cruden et al. [11].

Equation for pollen viability determination in *Passiflora edulis* Sims

$$\text{Pollen Viability} = \frac{\text{Amount of Viable/Unviable Pollen}}{\text{Total Amount of Pollen}} \times 100 \quad (1)$$

2.4 Determination of Pollen/Ovule Ratio

Ovule counting was performed on the same flowers used for pollen viability. A longitudinal section was performed on the ovary and all present ovules were counted. For counting, the ovules were deposited on checkered slides and observed under a branded optical microscope and above-mentioned models. The pollen/ovule ratio was determined by dividing the total amount of pollen by the total number of ovules in the flower bud adjusted by the equation of Eqn. 2. The reproductive system of the species was determined according to the classification proposed by [11].

Equation for determining the pollen/ovule ratio in *Passiflora edulis* Sims

$$P/O \text{ Ratio} = \frac{\text{Total Amount of Pollens}}{\text{Total Amount of Ovules}} \quad (2)$$

2.5 Data Analysis

The parameters evaluated were the number of viable and unviable pollen grains per flower, the number of ovules and floral character biometrics estimated by simple descriptive analysis, represented by means and standard deviation using the R version 3.4.1 statistical software [12].

3. RESULTS

The flowers of this species have a color ranging from white to lilac, had an average of five sepals and petals. They presented an average of 208 fimbria and the ovary has a coloration ranging from yellow to light green. The androecium is formed by five stamens containing in each one an anther, the fillets are of short length (Table 1), inserted below the ovary. The gynoecium is

formed by three stigma, style and ovary, with an average height of 16,5mm. For greater pollination success, the stigma should be located below the anthers, as this position facilitates contact with the pollinator; however, the anthers of this species positioned below the stigma, this possibly makes it difficult to self-pollination.

Passion fruit (*Passiflora edulis* Sims) flower buds are large, hermaphroditic, in other words, composed of gynoecium, which is the female reproductive organ, and the androecium, which is the male reproductive organ. It has five versatile dorsifixed anthers with a large amount of yellow colored pollens, which are heavy, which makes wind pollination difficult. It has an upper ovary with three stigmas and consists of a large number of ovules. (Table 2).

It was observed high amount of viable pollens present in the flower, representing 99.8%, and unviable represented 0.2%, values obtained using the colorimetric method, with the aid of Alexander's reactive, Fig. 2.

Table 1. Floral biometrics of *Passiflora edulis* Sims

<i>Passiflora edulis</i> Sims			
Flower buds	Reproductive organs	Length (mm)	Diameter (mm)
10	Ovary	0,16±0,01	0,10±0,01
	Androecium (stamens)	10,9±0,06	-
	Gynoecium (style)	16,5±0,23	-

Table 2. Viable and unviable pollen grain quantities and ovules of *Passiflora edulis* Sims

<i>Passiflora edulis</i> Sims			
Flower buds	Pollen		Ovules
	Viable	Unviable	
10	32136±3183	58±20	340±120

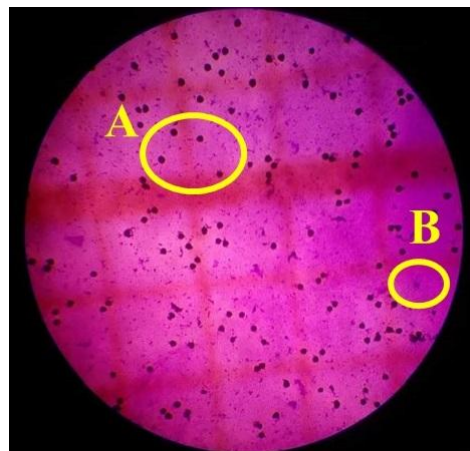


Fig. 2. (A) Viable pollens and (B) Unviable pollens of *Passiflora edulis* sims stained with Alexander reactive

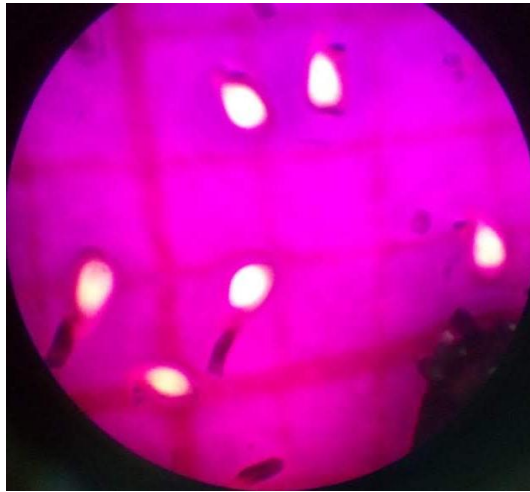


Fig. 3. Ovules of *Passiflora edulis* stained with Alexander's reactive

The pollen/ovule ratio for this species was 94.7/1; the data indicate that for each ovule present in the flower, there are approximately 94.7 pollen grains for fertilization. According to the classification proposed by [11], the reproductive system of the species points to facultative autogamy. The ovules are represented in Fig. 3.

4. DISCUSSION

According to [13], the petals are generally colorful and attractive to pollinators and other floral visitors. They also provide protection for the two main whorls in the bud stage. Passion fruit flowers have anthesis between 12 and 13 hours, but after a period of 5 hours, they undergo a wilting process, and the petals and sepals change color [14]. The fimbria are constituted by several filiform filaments distributed in one or more series, forming the corona that is an important structure in the botanical classification of *Passiflora* species, because they have attractive colors and have the function of attracting insects [15].

According to Esashika et al. [15], the ovarian diameter averages of some passion fruit species vary between 13 mm and 52 mm, while the height varies between 13 mm and 90 mm. One of the characteristics that most contributes to the genetic variability of passion fruit species is the ovary height, which is 25% important [16]. According to [17] the most efficient pollinating agents of passion fruit are the mamangavas (*Xylocopa* spp.), some smaller insects can collect nectar, but without pollinating the stigma.

Hermaphroditism is the predominant reproduction system in flowering plants, of which over 90% have both reproductive organs – male and female [18]. Having two sexes in a flower increases pollen removal and deposition efficiency by pollinators, although it is generally suggested that hermaphrodite plants are under selective pressure to separate male and female functions to prevent inbreeding depression [19].

In this case, some strategies for separation of sexual functions are common, such as, for example, dicogamy or temporal separation [20, 21], herkogamy or spatial separation [22] and genetic-physiological or self-incompatibility separation [23].

Studies on pollen viability in hermaphrodite species provide important insights for understanding the reproduction of the species in the field, where the observed viability percentage may explain the abortion, or even the reproductive success of each species. A pollen viability below 70% is considered low, which can cause problems for the species, such as infertility. Low pollen grain viability values may indicate possible meiotic irregularities leading to different degrees of sterility [24].

Pollen viability is a limiting factor for the reproductive success of *Passifloraceae* [25]. They state [26] that pollen viability decreases linearly at the expense of collection time, with the highest results obtained by samples collected near the time of flower anthesis. Corroborating this, [27] described that *P. edulis* progressively loses its viability from 4 hours after anthesis. The flower buds used in the study were collected

closed at the beginning of the anthesis, around 11 o'clock, so we can observe the high viability in yellow passion fruit flowers.

They further state [24] that the coloring capacity with a given dye may vary between species. In their research using two types of dyes to estimate the pollen viability of *Psidium cattleianum* Sabine, they found that the use of acetic orcein caused viability overestimated, and that Alexander's reactive showed to be more efficient and reliable regarding the estimation of pollen grain viability through its coloring capacity in this species.

In the research conducted by [9], with *Passiflora edulis*, using the reagents, Alexander Reactive and 1% Acetic Carmine, the results show that there was no statistical difference between the reagents, they showed a high pollen viability > 80%, 88.11% and 88.84%, respectively, showing that the male gamete has a high fertilization capacity, these data corroborate this research with an estimated 99.8% pollen viability. Analyzes using the Alexander Reactive provide more accurate data on pollen viability since a differential staining of viable and non-viable pollens is obtained due to the simultaneous use of the chemical agents of its composition, which are double stained.

The pollen/ovule ratio observed in this study indicates that the species has a reproductive system by facultative autogamy, which is linked to the type of reproduction of the species. The pollen/ovule ratio is related to the pollination mode and the reproductive system of the species, considering that this plant can share being a species with mixed reproduction system, autogamy and allogamy.

According to [28] in studies on the reproductive system determination of *Passiflora cincinnata* and *P. quadrangular* genotypes, controlled cross-pollination provided the largest amount of fruit obtained, followed by open pollination, lastly self-pollination showing no fruit formation. These results show that these genotypes opt for allogamy, yet according to the authors, the lack of self-pollination approves self-incompatibility and genotype allogamy, which may make them adept at interspecific hybridization.

Yellow passion fruit is an allogamous plant, mainly due to its floral morphology and its heavy and sticky pollen grains, which makes anemophilic pollination difficult [29,30]. This

allogamy is reinforced by homomorphic and sporophytic self-incompatibility [31], although it is possible to find self-compatible plants [32].

Regarding *Passiflora edulis*, [14] state that the results of natural pollination carried out in two areas and two periods showed that in the rainy season there was no fruiting, however, there was 9.4% of fruit formation in the dry period, and there was no fruiting by self-pollination. Therefore, observed that passion fruit is a species that apparently, although hermaphrodite, opts for cross-pollination, being a self-incompatible species. In this work, we investigated the fertility of pollen and we did not test if the species is self-compatible.

In addition, passion fruit flowers have an attractive color, are showy, aromatic and abundant with nectar, causing strong attraction to pollinators. This fact favors cross-pollination, especially by insects [33,34].

According to Auler et al. [35], the pollen viability rate is an important factor for plant breeding, conservation and cultivation, because in allogamous species, such as the species under study, gene flow through pollen increases the possibility of formation of different combinations between alleles, and consequently of genetic variability. Second to [36] in studies with *Passiflora cincinnata* Mast based on morphoagronomic descriptors, within a single species of passion fruit, it is possible to find high variability for multiple traits of agronomic interest, as well as pollen viability between accessions, as well as fruit production, such traits should be evaluated in passion fruit breeding programs.

The data obtained in this study confirm the efficiency of Alexander's reactive dye to observe the pollen viability in passion fruit flowers, which indicates high viability of male gametes present in the flower. However, as regards the reproductive system of the species through the pollen/ovule ratio, it was observed that only by this classification, we cannot affirm the type of reproductive system, a field study will bring results that are more accurate on the reproduction of this species, which we cannot discard by research the facultative autogamy, probably performed by the species.

5. CONCLUSION

The *Passiflora edulis* Sims flower has a large amount of pollen grains present in the anthers,

which favors the sexual reproduction of the species.

Alexander's reactive dye was effective in staining the pollen grains of yellow passion fruit.

This species is optional autogamous for the estimate of reproductive tendency, suggested by the pollen/ovule ratio.

Passiflora edulis Sims flowers have uniform structures, with no morphological and biometric variations in the values of petals, sepals, anthers and stigma for the edaphoclimatic conditions of Paraíba swamp.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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