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Viabilidade polínica e biologia floral de Mandacaru (*Cereus jamacaru* (DC) (Cactaceae))

Pollen viability and floral biology of Mandacaru (*Cereus jamacaru* (DC) (Cactaceae))

Viabilidad del polen y biología floral de Mandacaru (*Cereus jamacaru* (DC) (Cactaceae))

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Paulo Marks de Araújo Costa
ORCID: https://orcid.org/0000-0001-6411-745X
Universidade Federal da Paraíba, Brasil
E-mail: paulomarksac@gmail.com
Vênia Camelo de Souza
ORCID: https://orcid.org/0000-0002-1847-9182
Universidade Federal da Paraíba, Brasil
E-mail: venia_camelo@hotmail.com
Ivan Sérgio da Silva Oliveira
ORCID: https://orcid.org/0000-0002-0046-146X
Universidade Federal da Paraíba, Brasil
E-mail: ivantecagro@gmail.com
Vagner Sousa da Costa
ORCID: https://orcid.org/0000-0001-7639-7727
Universidade Federal da Paraíba, Brasil
E-mail: vagnersousa14@gmail.com
Emerson Serafim Barros
ORCID: https://orcid.org/0000-0002-3295-4496
Universidade Federal da Paraíba, Brasil
E-mail: emersonserafim997@gmail.com

Resumo

A fertilidade do pólen é indicada pela viabilidade, a qual tem influência sobre o sucesso reprodutivo de plantas. O mandacaru é uma espécie nativa de porte arbóreo, com distribuição natural na Caatinga. Objetivou-se verificar a viabilidade do pólen, razão pólen-óvulo e a identificação morfológica da flor do mandacaru (*Cereus jamacaru*). Foram utilizados dez botões florais e cem anteras destes botões, as quais foram coradas com corante Alexander em lâmina de vidro e contabilizou-se os pólens viáveis e inviáveis, foram contabilizados a

quantidade de óvulos por botão floral e verificou-se a razão pólen/óvulo. O mandacaru apresentou viabilidade polínica de 99,41%, com média de 314.686 pólens em cada flor. Foram 356 pólens para cada óvulo, que indica o sistema reprodutivo da espécie para autógama facultativa e xenogâmica facultativa. A flor é hermafrodita, com gineceu medindo em média 160,9 mm, com média de 883 óvulos por flor, o androceu apresentou média de 577 anteras por flor, e 545 grãos de pólens por antera. O Mandacaru apresenta pólen com alta viabilidade, com flor hermafrodita, de tamanho grande, com quantidade elevada de anteras e grãos de pólens contidos nestas anteras.

Palavras-chave: Biologia reprodutiva; Caatinga; Espécie nativa; Xenogâmia.

Abstract

Pollen fertility is indicated by viability, which influences the reproductive success of plants. Mandacaru is a native tree species with natural distribution in the Caatinga. The objective was to verify the pollen viability, pollen per ovule ratio and the morphological identification of the mandacaru flower (*Cereus jamacaru*). Ten flower buds were used and one hundred anthers of these buds, which and stained with Alexander dye on checkered glass slide and viable and non-viable pollens were accounted amount of ovule per flower bud was counted the pollen/ovule ratio. Mandacaru presented viability of 99.41% the pollen, with an average of 314,686 pollens on each flower. There were 356 pollens for each ovule, this indicates the species reproductive system for facultative autogamous and facultative xenogamics. The flower is hermaphrodite, with gynoecium measuring an average of 160.9 mm, an average of 883 ovules per flower, the androecium averaging 577 anthers per flower, 545 pollen per anther. Mandacaru has high pollen viability, with large size hermaphrodite flower, with high number of anthers and pollen contained in these anthers.

Keywords: Reproductive biology; Caatinga; Native species; Xenogamics.

Resumen

La viabilidad del polen es indicativa de la fertilidad del polen, que influye en el éxito reproductivo de las plantas. Mandacaru es una especie nativa de tamaño arbóreo, con distribución natural en el bioma Caatinga. El objetivo era verificar la viabilidad del polen, la relación polen-huevo y la identificación morfológica de la flor de mandacaru (*Cereus jamacaru*). Se utilizaron diez yemas florales para la viabilidad del polen y cien anteras de estas yemas, que se tiñeron con tinte Alexander en un portaobjetos de vidrio y se contaron pólenes viables y no viables, se contó el número de huevos por flor y relación polen / huevo. Mandacaru

mostró una viabilidad de polen de 99.41%, con un promedio de 314,686 pólenes en cada flor. Había 356 pólenes por cada huevo, lo que indica el sistema reproductivo de la especie para el autogamo facultativo y el xenogamo facultativo. La flor es hermafrodita, con ginecium midiendo un promedio de 160.9 mm, con un promedio de 883 huevos por flor, el androceu mostró un promedio de 577 anteras por flor y 545 granos de polen por antera. Mandacaru tiene una alta viabilidad de polen, con una flor hermafrodita de gran tamaño, con una gran cantidad de anteras y granos de polen contenidos en estas anteras.

Palabras clave: Biología reproductiva; Caatinga; Especies nativas; Xenogamia.

1. Introduction

The Cactaceae family has species with the capacity to develop in different ecosystems, but the largest representation occurs in semi-arid and arid climate. Cactaceae are the second family of angiosperm with endemic species from warm and dry climate regions (Taylor & Zappi, 2004). The largest distribution centers of Cactaceae are the United States, Mexico, Southeast and Northeast of Brazil. Brazil is the third largest distribution and diversity center. There are 260 species and 39 genera, of which 187 are endemic (Casas et al., 2014).

Mandacaru (*C. jamacaru*) is classified in the family Cactaceae, subfamily Cactusidae, Cereae tribe; has a natural distribution in the Brazilian semiarid, with great ecological, economic and social importance for Caatinga and human populations (Lucena et al., 2015a). The mandacaru grows in warm climates with stony, shallow soils (Pereira et al., 2018).

Pollen viability confirms pollen fertility, which has a direct influence on fertilization and consequently on the reproductive success of plant species, since the amount of seeds and the of fruits formed depend on the of viable pollens (Rigamato & Tyagi, 2002). Studies on pollen viability are important because provide basic information for practical application in conservation plans, taxonomic, palynological, ecological and population and ecosystem management studies, highlighting the male potential of plant reproduction.

Pollen viability is evaluated by staining methods and direct pollen counting; there are several varieties of dyes that have been used. The most commonly used dyes to test pollen viability are nuclear and vitas are Alexander, Acetocarmine, Tetrazolium Salts and 11 aniline Blue in lactophenol (Frescura et al., 2012).

Studies on native species are of utmost importance and urgency, especially Caatinga cactus species. It is necessary to search for information that can support the conservation, preservation and sustainable management in the Caatinga (Lucena et al., 2015b).

The aimed to verify the pollen viability, pollen per ovule ratio and morphological identification of mandacaru flower (*Cereus jamacaru* (DC)).

2. Material and Methods

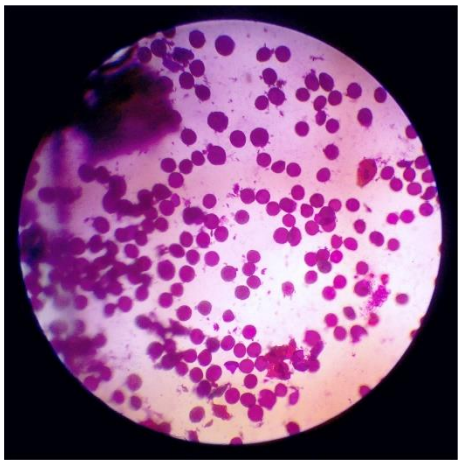
A population of mandacaru (*C. jamacaru*) was selected from a fragment of Caatinga forest located in the city of Bananeiras, Paraíba, Brazil. The vegetal resources of this area are constituted by deciduous species; being chosen for presenting characteristics of forest formations of Caatinga in different levels of ecological succession, as well as in all previously recorded the natural occurrence of Cactaceae with a population of mandacaru (*C. jamacaru*) (De Araújo Costa et al., 2020).

Ten flower buds of the species were collected, one flower bud per plant, and stored in 70% alcohol for up to 24 hours. The analysis of pollen and ovules grain count, pollen viability and morphological identification of the flower were made at the Campus III Biology laboratory of the Federal University of Paraíba, Bananeiras, Paraíba, Brazil. Following the active methods proposed by Pereira et al. (2018).

To pollen viability, the Alexander colorimetric reactive method (Alexander, 1980) was observed, in which ten anthers were removed from ten flower buds, which were macerated, one at a time, in a checkered glass slide containing 1ml of distilled water and added the Alexander reagent (Alexander, 1980), observed under BEL®photonics model 0928896 optical microscope, with lens approaching 4/0.10 (4x), the pink-colored pollen grains intense were considered viable (Figure 1) and those that were light blue were not viable (Kearns & Inouye, 1993); STARFER® model 03579409 manual number counters were used to perform the counting.

The average amount of pollen per anther and flower was determined by quantitative means. The viability was represented as a percentage, following the equation in Figure 2, the amount viable pollen was were divided by the number of total pollen per flower bud and multiplied by one hundred, thus totaling the of viable pollen in each evaluated flower.

Figure 1. Pollen of *Cereus jamacaru* stained with Alexander reactive, observed in optical microscope.



Source: Research data.

Figure 2. Equation to determine the amount of viable pollen per flower.

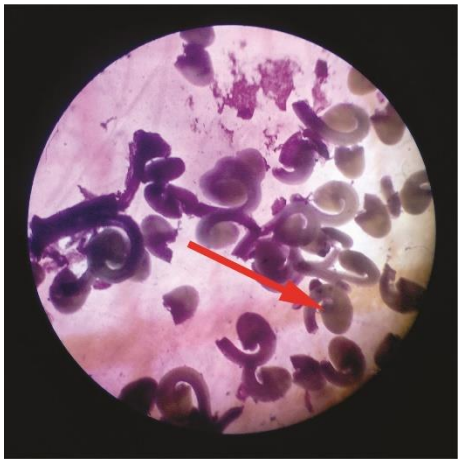
$$\text{Pollen viability} = \frac{\text{Nº of viable pollen grains}}{\text{Nº of total pollen grains}} \times 100$$

Source: Authors.

Ovule counts were performed on the same flowers used for pollen viability. A longitudinal section on the flower was performed and only the ovules on one side of the ovary were counted, multiplied by two to obtain the total amount of ovules present in the flowers.

For counting, the ovules were deposited on slides checkered glass and added 1ml of distilled water, were observed in an optical microscope of the aforementioned brand and model, as indicated by the red arrow in Figure 3 which shows the ovule, these were counted with the aid of numeric counters of the aforementioned brand and model. The pollen/ovule ratio was determined according to the equation in Figure 4, in which the total of pollen an anther was used and subjected to division by the total number of ovule from a floral bud, the value of the division was multiplied by total of anthers of a flower bud, thus obtaining the total amount of pollens for each ovule per flower bud, after the result of the equation shown in Figure 4, the reproductive system of the species determined according to Cruden, (1977) was determined.

Figure 3. Ovules of *Cereus jamacaru* observed in optical microscope.



Source: Research data.

Figure 4. Equation to determine pollen quantity for each ovule per flower.

$$\text{P:O Ration} = \frac{\text{Nº of pollen grains in an anther}}{\text{Nº of ovule}} \text{ x Nº of anther}$$

Source: Cruden (1977).

For the morphological identification of the flower was used Lotus digital caliper, Marte® analytical scale model AY220 and graduated ruler of thirty centimeters. The dimensions of the external floral elements observed in the study were: length, inferior diameter (base of the flower bud) and superior diameter (apex) and weight. The biometry of the dimensions of the floral characters of the internal part of the flower was also performed. From the length of the gynoecium of ten pre-anthesis flower buds, for the average of the androecium, average data of 300 stamens length were collected, being 30 stamens of each flower bud pre-anthesis. Data per to morphology analyzes were represented as means and standard deviation with the aid of RStudio© Version (1.3.959/2020).

3. Results and Discussion

Mandacaru flower buds (*C. jamacaru*) showed pollen viability of 99.41%, anthers average with 577 anthers per flower, average 545 pollen per anther and average pollen per flower was 314,686.

According to Rocha et al. (2007), *Pilosocereus tuberculatus* (Werderm.) Byles & Rowley, a cactus species that is also endemic to the Caatinga, has a pollen viability of 98% with from 1,200 pollen per anther. The of pollen per ovule indicates a possible strategy of these species to ensure reproduction in the Caatinga. According to Souza et al. (2002), pollen viability above 70% can be considered high, from 31 to 69% considered medium and up to 30% considered low, ie *Cereus jamacaru*, as well as *Pilosocereus tuberculatus* have high pollen viability.

The flower had an average of 883 ovules, and a pollen/ovules ratio of 356/1, ie 356 pollen for each ovule, and according to Cruden's classification (1977) the reproductive system of mandacaru (*C. jamacaru*) can be classified into Facultative Autogamous and Facultative Xenogamy.

Plant species with reproductive system classified as facultative Autogam, produce fruits and seeds by self-pollination and spontaneous pollination (Kiill and Simão-Bianchini, 2011). Facultative xenogamy occurs when the pollinator visits the flowers of various plants, making contact with the male and female floral structures, thus generating cross-pollination, but this may or may not happen, according to Mandujano et al. (2014). Species with smaller the number of pollens are likely perform self-pollination, most likely due to the large amount of pollen present in the flower. Mandacaru has greater reproductive success by cross-pollination. This is a beneficial relationship for the species, since the cross-pollination favors the reproduction of the species in the field and avoids high homozygous rate in individuals of the population. the value

The Cactaceae family, gênero *Melocactus*, genus, under pollen/ovule ratio prevails the reproductive system by facultative autogamous (Nassar et al., 2007). Amount of pollen for each ovule is an accurate and inexpensive technique used to evaluate the types of reproductive systems of plant (Cruden, 2000). It is extremely necessary to detect the amount of pollen for each ovule of plant, as it is largely related to floral aspects and pollination (Lenzi et al., 2005).

The species has hermaphrodite and tubular flowers, usually located in the apical and subapical region of the plant and tends to horizontal orientation. The color of the floral tube is

green, large in size and heavy weight. The results of external biometrics are presented in Table 1.

Table 1. External floral biometrics of *Cereus jamacaru*.

Flower Buds	Length (mm)	Bottom Diameter (mm)	Superior Diameter (mm)	Weight (g)
10	214,9 ± 14,42	8,83 ± 0,51	19,71 ± 1,35	76,21 ± 11,01

Source: Research data.

According to Larios-Ulloa et al. (2015), flower buds of three species of *Mammillaria*, in the Cactaceae family, being *M. polythele* Mart. subsp. *Polythele*, *M. crinita* DC. subsp. *Crinita* e *M. uncinata* Zucc. ex Pfeiff. present length of 38.6, 128.4 and 166.6. Some cactus species genus *Pilosocereus*, *Melocactus*, *Mammillaria* and *Cereus* have hermaphrodite flowers (Rocha et al., 2007).

Phenological studies are of great importance to establish plans for conservation of species, being established for example, floral characteristics such as color, size and position of structures, as well as anthesis time and pollination syndrome (Aona et al., 2006). Another reproductive influence on species is plant size. It has a significant relationship with number of anthetic flowers and flowers and the amount of fruits produced per plant (Larios-Ulloa et al., 2015).

The flowers of mandacaru (*C. jamacaru*) have green-colored sepals and white petals, have gynoecium and androecium, in which gynoecium is the female organ with the inferior ovary, ovules, stylet and stigma. The androecium is the male organ, formed by stamens, which is composed by the fillet and the anther, the pollen are stored in pollen sacs located in these anthers, and the set of gynoecium and androecium form the reproductive structures of the flower. The biometrics results of the inner part of the flower are presented in Table 2.

Table 2. Floral biometrics of *Cereus jamacaru*.

Variable	Length (mm)	Average amount per flower
Sepals	-	23,9 ± 8,25
Petals	-	20,7 ± 5,89
Anthers	-	652 ± 64,92
Gynoecium	160,9 ± 32,87	-
Stamens	72,3 ± 6,48	-

Source: Research data.

Each flower has a set of morphological that, in a way, directs the floral visitor. According to Vieira & Fonseca (2012), morphology and floral biology are closely related to pollination and reproduction. This set of morphological characteristics or floral attributes that are common to certain groups of pollinators is termed Floral Syndrome (Faegri & Van Der Pijl, 1979). *Cereus jamacaru* has attractive characteristics for floral visitors, being a large flower full of pollens and strong odor. Its nocturnal anthesis shows that due to its floral morphology, its pollinator is probably the bat, and the presence of secondary pollinators is not dismissed.

4. Final Considerations

Cereus jamacaru presents high pollen viability, with 99.41% of viable pollens; The pollen/ovules ratio of 356/1 and the reproductive system of *Cereus jamacaru* was classified into facultative autogamous and facultative xenogamy. The flower of *Cereus jamacaru* is large sized, hermaphrodite, with high amount of anthers and pollen grains contained in these anthers.

The results presented in this paper can support long term studies about the ecological aspects of *Cereus jamacaru*, in which knowledge about the reproductive characteristics of plants can provide important information for the development of conservation strategies and management of species in natural environment.

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Percentage of contribution of each author in the manuscript

Paulo Marks de Araújo Costa – 30%

Vênia Camelo de Souza – 25%

Ivan Sérgio da Silva Oliveira – 15%

Vagner Sousa da Costa – 15%

Emerson Serafim Barros – 15%