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Evaluation of *Dypsis lutescens* (H. Wendl.) pollinic viability by certain colorimetric tests

L. P. N. Ramos, Z. M. da S. R. Prestes, E. R. Ramos, C. B. M. Farias, W. de A. Macedo, V. dos S. de Mello, I. V. Karsburg

Universidade do Estado de Mato Grosso - Campus de Alta Floresta - MT

Author for correspondence: leila_pereiramos@hotmail.com

Abstract: *Dypsis lutescens* is predominant in regions with tropical, subtropical and equatorial climate. Areca, has considerable commercial value in Brazil, is highly cultivated in large scale for landscaping and decoration purposes. The objective of this work was to evaluate the viability of *D. lutescens* pollen through the staining test, using Lugol 2%, 2% acetic Orcein, 1% Malachite Green, Methylene Blue, 1% Fuchsin, 5% Giemsa and the Reactive of Alexander. For the tested dyes, the viability of the pollen was determined by the staining aspect where it was considered feasible the protoplasm pollen with defined staining and unviable the pollen with stained exina and protoplasm absence. To prepare the slides, the floral buds were cut diagonally with the scalpel to remove the anthers, then the maceration process of the anthers was made with a glass stick, to release the pollen, with a drop of 0.5 mL of each dye, covering the material with a coverslip, after that the slide was observed in an optical microscope at 40x, using the scanning procedure, counting 300 pollen per slide, with 5 repetition for each dye, totalizing 1500 grains. In the present study, it is possible to conclude that Areca *D. lutescens* palm, although presenting low pollen viability percentages, for some dyes to which it was tested, Acetic orcein 2% Green malachite 1% Methylene blue and Guiemsa 5% showed good results to distinguish viable pollens from unviable ones, being these dyes indicated to test the pollen viability of the species.

Keywords: Arecaceae, Dyes, Palm tree.

Introduction

There are in Brazil many plant species that are used to ornamental purposes, small and medium species are the most sought after, because the backyards are increasingly reduced (SIVEROL, 2014).

Arecaceae family that is originally from África, is between the plants that are often used for this purpose. In this family is found Areca from *Dypsis lutescens* gender which is predominant in regions with tropical, subtropical and equatorial climate. Areca is a bush that can reach from 4 to 12 meters height, it creates clumps with green and yellow palm heart, curved leaves with yellow fruits and numerous ovoids, in the summer it has a wide fruit production (SILVA, 2002).

Because of your beauty, Areca has considerable commercial value in Brazil, it is highly cultivated in large scale for landscaping and decoration purposes. The flowers of this species appear in large amounts, they are small, aromatic and has white color. Even if it has tropical characteristics, it is better to keep this plant in half

shade, avoiding this way burnt and yellow leaves (LORENZI, 2001).

The pollinic viability study is an instrument very used to verify pollen grain pollinic viability of a specific species. These studies are one of the ways of obtaining information about the biology of the species and the genic flow of it, which are fundamental to genetical enhancement (BRAGA, 2018).

The pollinic viability study is very important to understand the species reproductive aspects, because it produces few fruits if compared to flowers production. These studies are important to preserve the species, also for cultivating ornamental and with commercial value species. It is still an important instrument for genetical enhancement studies (LEITE, 2018).

Plant breeding studies pollinic estimation of one species, evaluating the pollen grain viability, being possible to know if it will germinate the flower stigma, which is a decisive step when related to the plant fertility (BIONDO, 2001).

Pollinic viability can be determined through chemical dyes that react with components present in mature pollen grains. For pollinic viability tests it is important to use more than one dye, because it can present a result closer than the obtained by *in vitro* pollen germination (HISTER, 2016). Therefore, the goal of this research was evaluate *D. lutescens* pollen viability, through staining test using 2% Lugol, 2% Acetic Orcein, 1% Malachite green, Methylene Blue, 1% Fuchsin, 5% Giemsa and Alexander Reactive.

Methods

The study was made in the Cytogenetic and Plants Tissue Culture Laboratory, at UNEMAT Alta Floresta campus.

Flower buds in pre-anthesis were used to conclude the pollinic viability of *Dypsis lutescens*. It was obtained in geographical coordinates in Alta Floresta-MT town (Table 1).

Table 1. Identification of accesses, collecting spots of Areca flower buds in pre-anthesis and GPS data

Population	Collecting spots	Coordinates
Pop.1	Alta Floresta - MT	S: 09°51'25.2108 "W: 56°04'8.58"
Pop. 2	Alta Floresta - MT	S: 9°51'25.3656 "W: 56° 4'8.4504"
Pop. 3	Alta Floresta - MT	S: 9°51'25.2252 "W: 56°4'8.3028"

For testing *D. lutescens* pollen grain viability, the following dyes were used: 2% Lugol, 2% Acetic Orcein, 1% Malachite green, Methylene Blue, 1% Fuchsin, 5% Giemsa and Alexander Reactive. The flower buds were collected and cut crosswise to remove the anthers, macerating them in the slides with a glass stick releasing this way the pollen grain, then one drop of dye is used and the following step is to cover the material with a coverslip. Five slides with each dye were made respectively, and the pollen grain color was observed (viable and unviable).

Pollinic viability was determined by the pollen grain staining capacity, being considered as viable the pollen that presented color of exina and intine or protoplasma well defined, and unviable those that presented colored exina and protoplasm absence. In order to obtain pollen grain samples, scan methods were used, evaluating 300 pollen grain per slide and observed in optical microscope at 40X.

After counting both treatments, viable pollen percentage was calculated through the formula: Colored grain number/Counted grain number*100, compared by Scott-Knott test and by Tukey, both in 5% probability level by R, versão 3.3.2 program (R CORE TEAM, 2016) with the help of ExpDEs kit, 1.1.2 version (FERREIRA et al., 2013).

Results and discussion

On table 2 data studies of *D. lutescens* pollen viability were observed, using the following dyes: 2% Lugol, 2% Acetic Orcein, 1% Malachite green, Methylene Blue, 1% Fuchsin, 5% Giemsa and Alexander Reactive. It shows the dyes that obtained the highest percentage of fertile pollen were 1% Malachite green and Methylene Blue for the population 1, for the population 2 the dyes that showed the highest pollen viability percentage were, 2% Acetic Orcein, 1% Malachite green, Methylene Blue and 5% Giemsa. For population 3, the most effective dyes to distinguish the viable and unviable

pollen were, 2% Acetic orcein, 1% Malachite green and Methylene Blue. Alexander Reactive obtained the lowest pollen viability index for the 3 species population.

Using staining method in a study, Macedo et al., (2016) obtained similar results when using Reactive Alexander with *Alpinia zerumbet* species, concluding that this specie viability is considerably low with the use of this dye, indicating 53% of viable pollen.

According to Oliveira et al. (2010) when testing Alexander dye and 2% acetic orcein to estimate the pollen viability of *Euterpe oleracea* and *Euterpe precatória*, Alexander dye was effective, and 2% acetic orcein was not so efficient to distinguish the viable pollen of unviable ones. Both species had no differences as for pollen viability, presenting overall average of 94% of viability. *Euterpe oleracea* obtained 96% average and *Euterpe precatória* 92% average.

According to Oliveira (2015) the pollinic viability measurement, refers to analysing viable and unviable pollen at the meiosis end. This type of analysis can be made by staining methods. Between the methodologies used for this evaluation type, the staining methods were used for this purpose. Temperature variation can reduce the pollen and its viability and can result in problems during fertilizing process.

Dafni et al. (2000) related that the pollinic unavailability in the species, can occur by countless reasons, being them genetical or not, endogenous and exogenous from the pollen grain age, physical damages, morphological formation, microsporogenesis characteristics, moisture, temperature, and another reason for the high pollinic unavailability can be caused by meiotic irregularity.

According to Souza (2002), the viability values above 70% are considered high pollen viability, from 31 to 69% are considered medium and 30% as low. Based on this statement and according to the results

that were obtained in this study, the evaluated individuals presented pollinic viability percentages considered low, medium and high.

Neto et al. (2006) also evaluated the average of low pollinic viability in different *Solanum paniculatum* population species, they say that this event may be associated with the flower bud development and possibly related to the time of material collecting.

For the 3 *D. lutescens* populations of this study, solution of 2,3,5 triphenyltetrazolium (TTC) in two concentrations were used: 0,075% and 0,30% exposed during four different times (6, 12, 18 and 24 h). This test had no significant results. The reason for this pollinic unavailability may occur because this specie reproduce by apomixis and presents exotic characteristics. Happening this way the asexual reproduction, originating fertile seeds and low viability pollen (CZAPIK, 2000). According to the need or not of pollination, apomictic species are classified in autonomous species (ASKER et al., 1992). In face of this result, it is emphasized that *D. lutescens* palm, has autonomous apomictic characteristics, without need of pollination to develop seeds, with low viability pollen grains.

On table 1 it is observed the *Dyopsis lutescens* pollen, tested with different dyes: 2% Lugol, 2% Acetic Orcein, 1% Malachite green, Methylene Blue,

1% Fuchsin, 5% Giemsa and Alexander Reactive, all tested dyes showed they are able to distinguish viable and unviable pollen. It is showed that in the staining process of viable pollen, it can indicate the viability of species by the difference between staining viable pollen and unviable pollen, which the cellulose present in the cell wall is stained. According to Alexander (1980), because it does not present protoplasm, the unviable pollen grains stain in a greenish tone.

Analysing *Theobroma cacao* species, Cabral et al. (2013) related that the staining using Lugol happens between an iodine and starch reaction, resulting in light brown pollen to viable and light and transparente staining to unviable. It occurs because of the absence of starch, coinciding with the pollen staining results of this study with Areca palm.

According to Gomes et al. (2013) study, with *Mauritia flexuosa* species, 2% Lugol obtained 96,84%. The *M. flexuosa* pollen have a large starch reserve, where 2% Lugol react with this starch. In the same study Alexander Reactive was used obtaining 95,44% of pollinic viability.

In Alexander's method (1969), the nucleus of viable pollen grain reacts with Fuchsin and the viable pollen stains in pink, while the unviable grains stain in green.

Table 02. Viability Percentage of Areca pollen grain stained with different cytochemical methods.

Dyes	Populations		
	01	02	03
2% Lugol	22,13Ac	32,33Bd	7,86Be
2% Acetic Orcein	22,00Cc	83,13Ab	69,26Bb
1% Malachite green	99,60Aa	81,60Bb	81,20Aa
Methylene Blue	96,46Ca	61,86Ab	60,86Bb
1% Fuchsin	39,80Ab	16,26Be	16,93Bd
5% Giemsa	23,53Ac	100,00Ba	44,00Bc
Alexander Reactive	18,66Ac	3,60Ae	35,40Bd
CV (%)	20,84		

Averages followed by the same lowercase letter in the column and uppercase in the row do not differ from each other by Knott test at 5% probability. CV (%) Variation Coefficient.

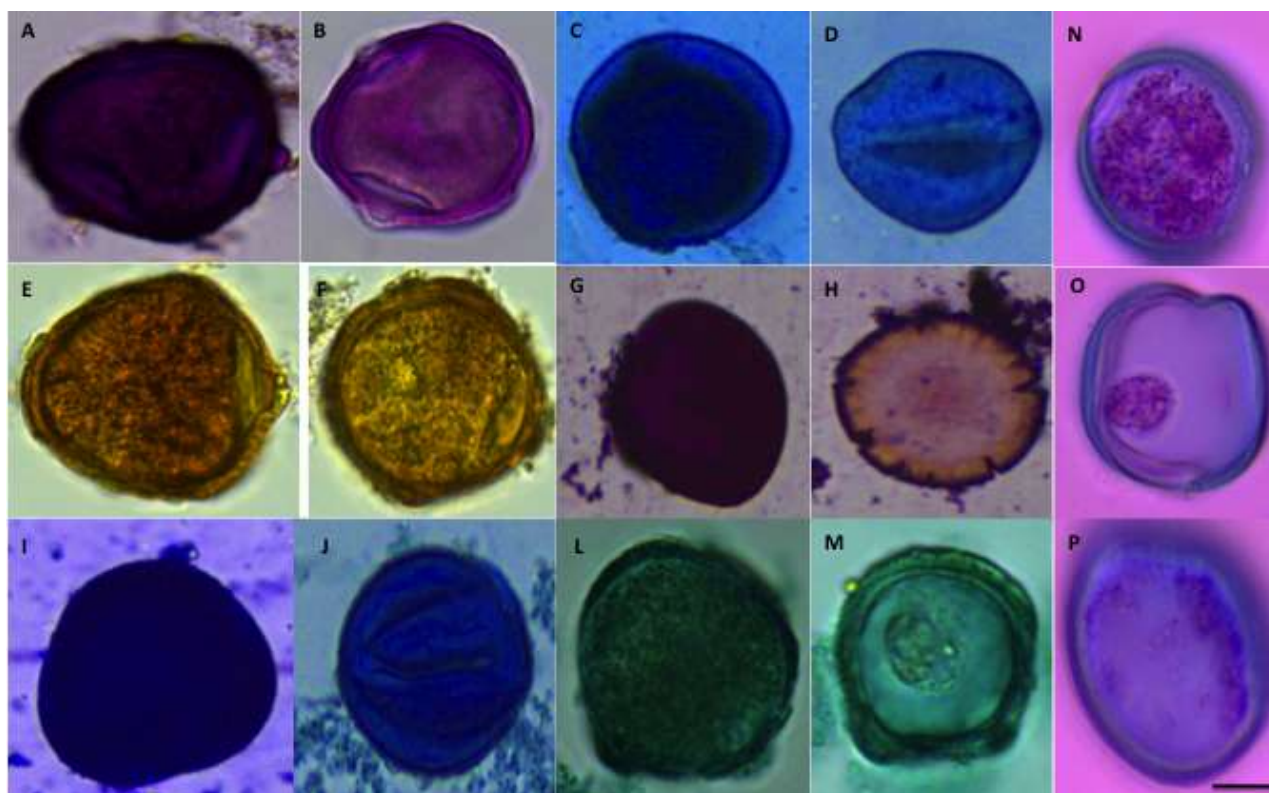


Figure 1. *Dypsis lutescens* pollen grains stained with different dyes: Grãos de pólen de *Dypsis lutescens* corados com diferentes corantes: Fuchsin 1% A) viable B) unviable. Giemsa 5% C) viable D) unviable. Lugol 2% E) viable F) unviable. Acetic Orcein 2% G) viable H) unviable. Methylene Blue. I) viable J) unviable. Malachite green 1% L) viable M) unviable. Alexander Reactive N) viable. O) unviable. P) unviable. Bar= 10 µm.

Conclusions

In the present study was possible to conclude that *Areca Dypsis lutescens* palm species, even presenting low percentage of pollinic viability for some dyes that were tested, 2% Acetic Orcein, 1% Malachite green, Methylene Blue, and 5% Giemsa were the dyes that better distinguished viable pollen form unviable ones, being these dyes indicated to test pollinic viability for the species under study.

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