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Evaluation of *Crossandra infundibuliformis* L. (Acanthaceae) polen viability based on 2,3,5 triphenyltetrazolium (TTC) and Lugol 2% colorimetric tests.

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Abstract: *Crossandra infundibuliformis* L. (Acanthaceae) is a shrub native to India. It is an important ornamental plant in horticulture because of its medicinal value, used to treat various diseases. The extract of its leaves contains properties with antifungal and antimicrobial activities. The objective of this work was to estimate the pollen viability of *C. infundibuliformis* found in three sites in the municipality of Alta Floresta, state of Mato Grosso. A solution of 2,3,5-triphenyltetrazolium (TTC) was used in the study at concentrations of 0,075% and 0,30% combined with the exposure of the material at four different times and Lugol 2% dye. A drop of each treatment was added to slides prepared using TTC, and then they were covered with a glass slide. In the Lugol 2% test, the technique of crushing the anthers for the preparation of the slides was used. In the estimate of pollen viability of populations 01, 02 and 03 of *C. infundibuliformis*, colorimetric tests (TTC and Lugol 2%) were efficient in distinguishing viable pollens from non-viable pollens. A gradual increase in the means was observed for both concentrations as the time of exposure was raised. The 0,30% concentration showed the highest averages of pollen viability. Nevertheless, the 0,075% concentration showed average percentages of viability at all times. According to the treatments to estimate the pollen viability of the species, the concentration of 0,30% in the 24-hour exposure time was the best treatment for the clear differentiation of the pollen grains. The viability averages of *C. infundibuliformis* were median for TTC concentrations and high for Lugol dye 2%.
Keywords: ornamental plant, estimate, dye.

Introduction

Crossandra infundibuliformis L. (Acanthaceae) is a shrub native to India. It has flowers with orange and monolobe corollas, commonly cultivated in full sun and half shade, usually in groups forming isolated clumps or in rows along live fences and walls (LORENZI et al., 2006).. *C. infundibuliformis* is an herbaceous ornamental plant, usually used in the formation of beds, whose diffusion is limited by the small production of seedlings (ALMEIDA et al., 2008).

According to Elamathi et al. (2011), *C. infundibuliformis* has in the extracts of its leaves, compounds with antifungal, antimicrobial and vermifuge activities. Due to its medicinal value, *C. infundibuliformis* is used in the treatment of various diseases (VADIVEL et al., 2016). The use of plant species for therapeutic purposes is a millenarian practice, still important nowadays as an alternative in curing and preventing diseases and as a source of raw material in the manufacture of medicines (FERREIRA et al., 2016). It is worth mentioning that scientific and popular knowledge is of great importance for the use of medicinal plants (SANTOS et al., 2017).

The study on pollen viability is essential for the breeding and conservation of plant species (CUSTÓDIO et al., 2011). The estimation of the pollen viability is vital for the analysis of the gene flow in plants since it evidences the male potential of the species production (FRESCURA et al., 2012).

In order to obtain better knowledge about the material studied, it is important that in a genetic breeding program, the pollen viability technique is used to obtain better knowledge about the material, thus progressing in the selection and prioritizing the best crosses (BRAMBATTI et al., 2016).

The utilization of tests with dyes has been used in the area of plant breeding as support to verify the fertility capacity of the pollen grain. In this context, the objective of this study was to estimate the pollen viability of *C. infundibuliformis* utilizing colorimetric tests using 2,3,5-triphenyltetrazolium (TTC) dyes in four different times (6,12,18 and 24 hours) and the Lugol 2%, evaluating the efficiency of the dyes on the species. This study may provide information and data on the pollen viability of this

species in order to contribute to the study and breeding of the species.

Methods

The work was carried out in the Laboratory of Cytogenetics and Culture of Plant Tissues at the State University of Mato Grosso, Campus of Alta Floresta, state of Mato Grosso. Thirty *C. infundibuliformis* L. floral buds were collected in the pre-anthesis stage, in the municipality of Alta Floresta, state of Mato Grosso (Table 1).

Table 1. Identification of accessions, pre-anthesis floral bud collection sites of *C. infundibuliformis* L. populations and GPS data.

Population	Collection site	Coordinates
Pop. 1	Alta Floresta - MT	South: 09º52'17,50 "West: 56º04'82,80"
Pop. 2	Alta Floresta - MT	South: 09º53'43,49 " West: 56º04'30,18"
Pop. 3	Alta Floresta - MT	South: 09º51'42,30 " West: 56º03'15,23"

At collection, the floral buds were cut transversally with the aid of a scalpel to remove the anthers, slightly macerating them with a glass stick to release the pollen grains in the 2,3,5-triphenyltetrazolium (TTC) solution at two concentrations (0,075% and 0,30%) that remained at four times (6, 12, 18 and 24 hours) of exposition to the dyes. The material was conducted in a controlled temperature environment at 30ºC.

Regarding the preparation of the slides, a drop of each treatment was deposited on the slide, and then the material was covered with a glass slide. For the analysis of the slides, the scanning method was adopted, where 300 grains of pollen was counted per slide with five replicates each, totaling 1500 grains of pollen for each treatment, observed through an optical microscope in the 40x objective.

Pollen viability was determined by the coloring capacity of the pollen grains, where pollens with exine and intine staining or red-colored protoplasm were considered viable and those that did not show any staining were considered non-viable.

By using the pollen counts, data were submitted to a comparison of means by the test of Tuckey at a 5% probability level through R software, version 3.3.2 (R CORE TEAM, 2016), with the aid of the ExpDes package, version 1.1 .2 (FERREIRA et al., 2013). Lugol dye data were graphically plotted on Sigma Plot software (version 11).

Results and discussion

The analysis of the viability of the *C. infundibuliformis* L. pollen grains allowed to observe a statistically significant difference between the means when the concentrations were compared (Table 2).

It was verified that the averages of pollen viability estimated by the two concentrations (0,075% and 0,30%) differed among themselves at all exposure times. For both concentrations, a gradual increase in the means was observed as the exposure time increased. The concentration of 0,30% presented the highest average viability of the pollen grains, with the highest percentage in 24 hours. The 0,30% TTC concentration showed no significant difference for all populations.

When working with the species *Lagerstroemia indica* L. at 6, 12, 18 and 24 hours, Mello et al. (2016) were also able to verify a gradual increase in the averages of the color of the pollen grains where the concentration of 0,30% at 24 hours of exposure was the best treatment to estimate the pollen viability of the species.

A difference was also found in the means tested with the 0,075% concentration as it presented an average percentage of viability at all times. Efficient methodologies with the use of tetrazolium (TTC) solution at low concentrations are important to optimize the application of the resources in the laboratories because they allow for a low cost and efficient analysis (SILVA et al., 2013).

The lowest percentages were observed at 06 hours for the two concentrations, 0,30% (59,33%) and 0,075% (41,66%). When testing the triphenyltetrazolium (TTC) dyes at four different times (6,12,18 and 24 hours) and Lugol 2%, Ramos et al. (2017) found that the pollen viability of the species *Momordica charantia* L. was also obtained at 6 hours for the two concentrations (0,30% and 0,075%).

Table 2. *C. infundibuliformis* L. pollen viability by 2,3,5 trifeniltetrazolio (TTC) staining at 0,075% concentration exposed at two temperatures.

TTC Concentration	Timers (horas)			
	6	12	18	24
0,075%	41,66b	44,66b	48,22b	51,55b
0,30%	59,33a	61,66a	70,13a	74,89a
CV (%)		31,45		

Means followed by the same upper-case letter in the rows and lower-case letter in the columns are not different from each other by the test of Tuckey at 5% significance level.

Data on Table 3 shows that Lugol 2% did not present significant difference among the means. The means of viability in this study were averages for concentrations of triphenyltetrazolium (TTC) and high for Lugol dye 2%.

In a work following the methodology similar to this one, Ramos et al. (2017a) used the species *Myrciaria cauliflora* where pollen viability, also estimated by Lugol 2%, was high for the populations 02 and 03, 72,66% and 77,00%, respectively, whereas the population 01 presented an average percentage with 67,66% of colored pollen.

Lima et al. (2015) in a study using the species *Malpighia emarginata* used Lugol and Alexander reactive, where Lugol was pointed with better precision in the results of pollen viability of the species.

Regarding the use of TTC (0.30% and 0.075%) and Lugol 2%, they were efficient in differentiating the coloration of viable pollen which was intensely stained and the non-viable were those not-stained or with reduced protoplasm in the pollen grain. Cabral et al. (2013) state that pollen viability is one of the processes that are responsible for the selection of genotypes for breeding programs and viable pollen grains influence the success of fertilization.

Ramos et al. (2017b) in a study testing different colorimetric methods, (2,3,5-triphenyltetrazolium (TTC) and Lugol 2%), obtained significant results using three *Myrciaria cauliflora* populations. The colorimetric tests were efficient in estimating pollen viability of the species, where the pollen presented precise distinct coloration, differentiating the viable from the non-viable pollen.

Table 3. Overall mean of pollen viability for *C. infundibuliformis* L. populations using TTC.

Populations	TTC		Lugol 2%
	0,30%	0,075%	
01	61,49a	42,58b	68,66a
02	65,58a	46,91ab	70,50a
03	63,91a	50,08a	73,16a
CV (%)	11,01	12,54	8,41

Means followed by the same upper-case letter in the rows and lower-case letter in the columns are not different from each other by the test of Tuckey at 5% significance level.

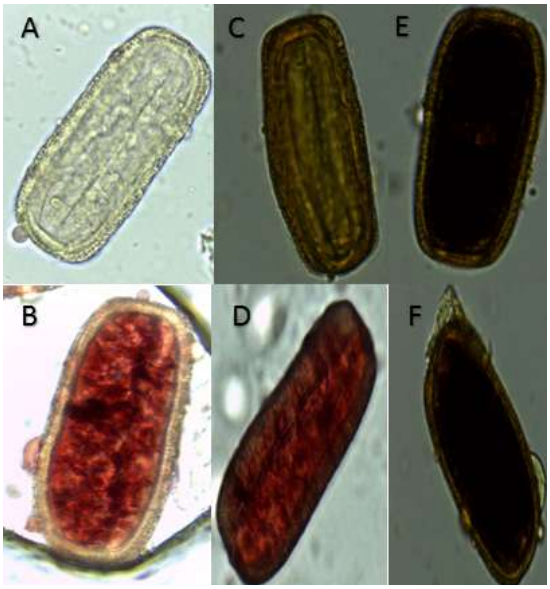

Figure 1. *C. infundibuliformis* L. Pollen grains stained with different dyes: 2,3,5 triphenyltetrazolium (TTC) a) non-viable, b) viable, c) non-viable and d) viable; Lugol 2% e) non-viable and f) viable. Bar = 10µm.

Conclusion

According to the data obtained with the three *C. infundibuliformis* L. populations, the colorimetric tests that used 2,3,5-triphenyltetrazolium (TTC) and Lugol 2% were efficient in estimating the pollen viability of the species.

The 0,30% TTC concentration at the 24 hour exposure time was the best treatment for the individual differentiation of the pollen grains. The viability averages of *C. infundibuliformis* L. were median for TTC concentrations and high for Lugol dye 2%.

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