Ornamental Horticulture
ISSN 2447-536X | HTTPS://ORNAMENTALHORTICULTURE.EMNUVENS.COM.BR/RBHO
SBFPO Sociedade Brasileira de Floricultura e Plantas Ornamentais

Scientific Article

Viability of pollen grains and stigma receptivity in Desert Rose

Cristiane Gonçalves Souza[1], Sabrina Maihave Barbosa Ramos[1], Silvia Nietsche[1]*, Clivia Carolina Fiorilo Possobom[1], Elka Fabiana Aparecida Almeida[1], Marlon Cristian Toledo Pereira[2]

[1] Universidade Federal de Minas Gerais, Instituto de Ciências Agrárias, Montes Claros-MG, Brazil
[2] Universidade Estadual de Montes Claros, Departamento de Ciências Agrárias, Janaúba-MG, Brazil

Abstract

Adenium obesum (Forssk.) Roem. & Schult., popularly known as desert rose, has become a valuable ornamental plant. In floriculture, the production of hybrids is prioritized. Hence, knowledge on floral biology and sexual reproduction of the target species is fundamental. The objectives of this study were: (1) to test sucrose concentrations and temperatures for *in vitro* germination of *A. obesum* pollen grains; (2) to identify the effect of temperature on the viability of *A. obesum* pollen grains; and (3) to evaluate the viability of pollen grains and stigma receptivity in pre-anthesis, at flower opening, and 72 h post-flower opening in three accessions of *A. obesum*. A significant relationship between temperatures and sucrose concentrations was observed in the *in vitro* germination test. The highest percentage of *in vitro* germination of pollen grains, 39.81%, was observed at an estimated temperature of 26.05 °C. Desert rose accessions maintained in biochemical oxygen demand (BOD) chambers at 30 °C during a 16-h light photoperiod showed faster flowering, and temperatures ≥ 25 °C induced pollen grain viability percentages above 69%. Temperature is one of the most important abiotic factors, influencing mainly in pollen germination, pollen tube growing and in efficiency fertilization. The ICA-wd accession stood out and can be considered a pollen donor in artificial pollination. The stigmas of flowers were receptive from a day before flower opening until three days after. The two parameters presented above, stigma receptivity and pollen viability, allow inferences about the appropriate time for successful pollination and subsequent fertilization in desert roses.
Keywords: anthesis, floriculture, sucrose, temperature.

Resumo

Viabilidade de grão de pólen e receptividade do estigma em Rosa-do-Deserto

Adenium obesum (Forssk.) Roem. & Schult, conhecida popularmente como rosa-do-deserto, tem se tornado uma opção valiosa para o setor de plantas ornamentais. Na floricultura, a produção de híbridos é priorizada, para isso, o conhecimento da biologia floral e da reprodução sexuada da espécie é fundamental. Objetivou-se com o presente estudo: (1) testar concentrações de sacarose e temperaturas na germinação *in vitro* de grãos de pólen de *Adenium obesum*; (2) identificar o efeito da temperatura na viabilidade de grãos de pólen de *Adenium obesum* e; (3) avaliar a viabilidade dos grãos de pólen e receptividade estigmática pré-antese, abertura floral e 72 horas pós-abertura floral em três acessos de *Adenium obesum*. Interação significativa entre temperaturas e concentrações de sacarose foi observada no teste de germinação *in vitro*. A maior porcentagem de germinação *in vitro* dos grãos de pólen, 39,81% foi observada na temperatura estimada de 26,05 °C. Os acessos de rosa-do-deserto mantidos em B.O.D. sob temperatura de 30 °C e fotoperíodo de 16 horas de luz apresentaram florescimento mais rápido e as temperaturas ≥ 25 °C indicaram porcentagens de viabilidade dos grãos de pólen acima de 69%. A temperatura é um dos fatores abióticos mais importantes, influencia a germinação dos grãos de pólen, crescimento do tubo polínico e pode afetar a fertilização. O acesso ICA-bd destacou-se e pode ser considerado como doador de pólen em polinizações artificiais. O estigma das flores encontrou-se receptivo desde um dia antes da abertura das flores até três dias após a flor abrir. Os dois parâmetros apresentados acima, receptividade estigmática e viabilidade polínica, permitem fazer inferências importantes sobre os momentos mais apropriados para a polinização e subsequente fertilização na rosa-do-deserto.
Palavras-chave: antese, floricultura, sacarose, temperatura.

*Corresponding author: silvia.nietsche@gmail.com

https://doi.org/10.1590/2447-536X.v28i1.2402
Received: Mai 31, 2021 | Accepted: Oct 07, 2021 | Available online: Nov 10, 2021

Area Editor: Carmen Silvia Zickel

Introduction

Adenium obesum (Forssk.) Roem. & Schult, commonly known as desert rose, belongs to the Apocynaceae family, and is native to sub-Saharan Africa (Plaizier, 1980). It is a shrub or small tree with a short stem, a thick base (caudex) that serves as a reserve organ, and spiral leaves, normally clustered at the end of twigs (Plaizier, 1980).

Similar to other representatives of the family, *Adenium* spp. flowers have exuberant beauty, marked by morphological complexity. They are actinomorphic or slightly zygomorphic; pentamerous, with a rounded or star-shaped corolla, funnel-shaped tube, five stamens with short filaments and long anthers with a sagittate base, forming a cone on the apex of the style (Plaizier, 1980; Colombo et al., 2018). The pistil exhibits two carpels that are coherent at the basis of the superior ovary and narrow into the single style which ends in a rounded, normally reel-shaped portion with bifid apex (Plaizier, 1980). The style apex can be divided into three regions, being below the basal collar, the place where the pollen grains normally germinate (Possobom et al., 2021). The pollen grains have an elliptical or spherical shape and the diameter may vary from 0.01 to 0.1 mm (Bennett and Willis, 2001). After fertilization, the apocarpous gynoecium gives rise to fruit formed by two polyspermic follicles united by the basal region (Plaizier, 1980; Colombo et al., 2018).

The species gained importance as an ornamental plant owing to its botanical and physiological aspects, including diversity of petal colors and floral arrangements, resistance to water stress, excellent adaptation in full sun environments, and perenniality (McBride et al., 2014).

Although desert roses can be easily propagated vegetatively (Colombo et al., 2018), sexual propagation is fundamentally important, especially for breeding programs, since it allows access to the great variability of the species (Colombo et al., 2018). Furthermore, reports show that plants propagated by seeds have a more developed caudex than those propagated vegetatively (Colombo et al., 2015; Colombo et al., 2018; McKain et al., 2018).

Considering the complex floral organization, which is a limiting factor to self-pollination, associated with the absence of natural pollinators and the possibility of self-incompatibility, propagation by seeds is still a challenge because it requires manual cross-pollination, which is not always successful. In addition, other factors may affect the success of pollination, including the existence of male or female sterility in cultivated plants (McLaughlin and Garofalo, 2002).

In this context, it is important to increase knowledge on pollen grains, since they are responsible for carrying the male gametes or their precursor to the stigmatic surface and conducting them through the growth of the pollen tube to the ovule, ensuring fertilization (Dafni, 1992). Therefore, understanding the aspects related to pollen grain viability and longevity, pollen–stigma interactions and the factors that may influence them is fundamental to guaranteeing crossbreeding efficiency.

Some growers have reported the efficiency of *A. obesum* crossbreeding when performed with pollen grains collected on the day of flower opening (unpublished data). However, there are no scientific data, except for the results of a study conducted by Avekin and Gaydarzhi (2016). According to the authors, the viability in *A. obesum* is 70% to 90% from the first to third day after anthesis.

The performance of pollen grains can be affected by different factors, extrinsic or intrinsic, in a variable way depending on the plant studied. Some studies have shown that environmental conditions during floral development can influence pollen grain fertility in different ways (Giovannini et al., 2017; Müller et al., 2016). Other studies have shown that factors related to the *in vitro* culture medium can affect pollen germination (Flores-Rentería et al., 2018; Impe et al., 2020). In addition, there is information relating the reproductive potential of pollen grains to the time they are removed from the anther, which involves seasonality or floral development stadium (Choudhary et al., 2014).

In addition to factors related to pollen grains, knowledge on details about stigma receptivity may be fundamental, since it may vary according to the floral development stadium (Yi et al., 2006) and may limit the success of pollination.

Temperature is the abiotic factor that most affects the reproductive aspects of flowers. Temperatures of 30 to 40 °C tend to shorten some phases of plant development, such as the reproductive phase. Thus, pollen quantity and morphology, anther dehiscence, and pollen wall architecture, as well as chemical composition and pollen metabolism may be influenced by elevated temperatures (Koti et al., 2005).

Although the desert rose is native to desert regions and is cultivated in different regions of the world, being, therefore, subjected to diverse environmental conditions, there are no scientific reports on which factors may affect pollen grain performance or stigma receptivity. Hence, this study was aimed at answering the following questions: 1) Is pollen viability in *A. obesum* influenced by temperature variation during floral development? 2) Do the *in vitro* pollen grain germination rate and stigma receptivity vary according to the floral development stadium? 3) Is the rate of *in vitro* pollen germination influenced by variations in sucrose concentration and temperature?

Materials and methods

Genetic material and experimental area

For the experiments, three accessions of *A. obesum* were selected and denominated as follows: (a) ICA-wd, an accession with white flowers and petal position in double arrangement; (b) ICA-rt, an accession with red flowers and triple petal arrangement and; (c) ICA-ps, an accession with purple flowers and single petal arrangement. Six 18-month-old plants of each accession and two finished blooms were selected. The plants were placed in 4 L plastic pots of 17 cm diameter containing Bioplant® commercial substrate, and maintained in a greenhouse with an anti-affecting screen and 20% shading.

Evaluation of pollen viability in plants subjected to different temperatures during floral development

Six *A. obesum* plants (accessions ICA-wd and ICA-rt) that showed the beginning of differentiation of the floral primordium (approximately 1.5 cm length) were selected and maintained, in pairs, in three BOD chambers (Limatec®) at controlled temperatures of 20, 25, or 30 °C in a 16-h photoperiod. Flower samples were collected on the first day of anthesis, and the pollen grains were removed with tweezers from all the anthers belonging to a single flower, mixed with a brush in a sterile Petri dish, and placed on slides with a drop of aniline blue dye. The slides were fixed with a drop of glycerin and then viewed under a microscope (Zeiss®) with a low-magnification objective (10×). Each slide was divided into four quadrants and 100 pollen grains were counted per quadrant. Pollen grains that were strongly stained blue were considered viable. The percentage viability was determined using the ratio of viable grains to the total number of grains evaluated on the slide.

A completely randomized experiment (CRE) was conducted with three treatments (20, 25, and 30 °C) and three repetitions. Each experimental plot was composed of one slide containing at least 400 pollen grains. The evaluated data were subjected to variance analysis, and when significant, to the F test, and the means were compared using the Tukey test at a 5% significance level.

Evaluation of *in vitro* germination of pollen grains and receptivity of stigmas in pre-anthesis, at flower opening, and 72 h post-flower opening

Flowers were collected from plants of the three accessions in pre-anthesis (a day before flower opening), at the day of the opening, and 72-h after flower opening, and sent to the Biotechnology laboratory. Pollen grains were carefully extracted from the anthers using a needle (0.45 × 13 mm, TKL brand) and inoculated in the center of a glass slide containing 150 µL of a standard culture medium (Brewbaker and Kwack, 1963): 1.27 mM Ca $(NO_3)_2 \cdot 4H_2O$, 0.87 mM $MgSO_4 \cdot 7H_2O$, 0.99 mM KNO_3, 1.62 mM H_3BO, and 100 $g.L^{-1}$ sucrose. For each flower, three slides were prepared and kept in a BOD incubator at a temperature of 27 °C in the dark for 6-h (Soares et al., 2015). The germinated pollen grains were counted under a microscope (Zeiss®) with a low-magnitude objective (10×), using 400 pollen grains per slide. Germination was considered effective when the pollen grains presented pollen tube length equal to or greater than the pollen grain diameter (Dafni and Firmage, 2000).

To evaluate stigma receptivity, the standard methodology of peroxidase activity using 3% hydrogen peroxide (H_2O_2) was used (Kearns and Inouye, 1993). A drop (approximately 0.05 mL) was deposited on the analyzed stigmas, and the reaction was filmed (Nikon D3400). The number of bubbles that emerged from the stigma within 30 s was counted (Dey et al., 2016; Biswas, 2017).

An entirely randomized design was used in a 3 × 3 factorial scheme, with three flower collection phases (pre-anthesis, at flower opening, and 72-h post-flower opening), three accessions (ICA-ps, ICA-wd, and ICA-rt), five repetitions to evaluate stigma receptivity, and three repetitions for pollen viability.

The data, when appropriate, were subjected to variance analysis, and when significant, to the F test, and the means were compared using the Tukey test at a 5% significance level. The data of germination percentage of pollen grains were transformed using logarithmic transformation (logX + 1) to obtain the homoscedasticity and normality of the data set.

Evaluation of *in vitro* germination of pollen grains taken from flowers at anthesis and subjected to different sucrose concentrations and temperatures

Flowers of the ICA-bd and ICA-vt accessions were collected on the first day of anthesis at 8 am. The pollen grains were extracted and inoculated as in second experiment, in adapted modified culture medium (Brewbaker and Kwack, 1963) with different concentrations of sucrose (0, 10%, 20%, and 30%), at pH 7.0. The slides, placed in Petri dishes with hydrated filter paper, were maintained in a BOD incubator at controlled temperatures of 15, 20, 25, and 30 °C for 6-h. After incubation, a drop of toluidine blue dye was added to count the germinated pollen grains under a light field binocular LED Digilab® microscope with a low-magnitude objective (10×). Each slide was divided into four quadrants and 100 pollen grains were counted per quadrant. The *in vitro* pollen grain germination percentage was estimated using the ratio between the number of germinated pollen grains and the total number of pollen grains counted on each slide.

The experimental design was entirely randomized in a 4 × 4 factorial scheme, with four incubation temperatures (15, 20, 25, and 30 °C), four sucrose concentrations (0, 10%, 20%, and 30%), and four repetitions. Each experimental plot was composed of one slide containing at least 400 pollen grains.

The data were subjected to variance analysis, and hence, it was possible to statistically determine some important precepts. The values for which the F test was significant, that is, greater than tabulated F, were analyzed, and a graph of response surface as a function of the independent variables (doses of sucrose and temperatures) was generated to determine the favorable conditions for each variable, using the R-Studio program. The data on pollen grain germination were transformed using logarithmic transformation (logX + 1) to obtain the normality of the data set.

Results and discussion

The plants cultivated at 30 °C took, on average, 15 days from the beginning of floral development to flower opening. At 25 °C, they took an average of 22 days to flower opening and at 20 °C, 28 days. At 20 and 25 °C, the plants presented floral abscission and yellowing of leaves. At 25 and 30 °C the viability of pollen grains was higher than 69% (Figure 1).

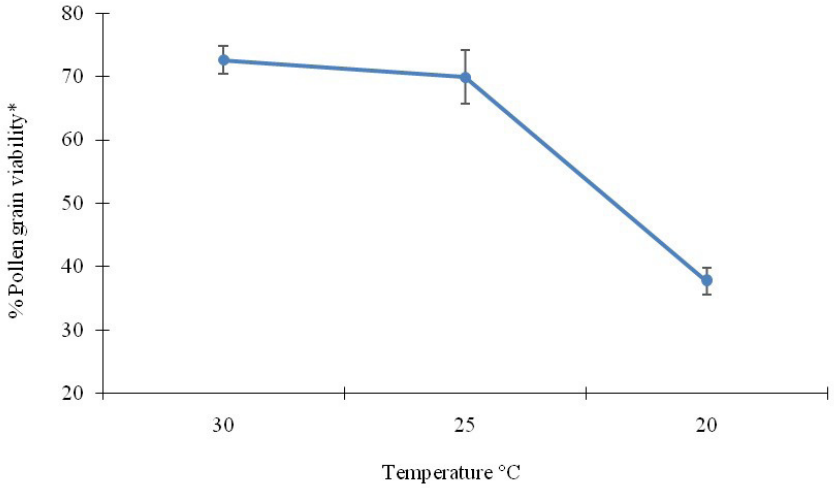

Figure 1. Percentage of pollen grain viability of *Adenium obesum* plants submitted to different temperatures (20, 25, and 30 °C) during floral development. *Significant at 5% by F test.

The present study showed that the temperature of 20 °C in a 16-h photoperiod considerably affected pollen viability, and there is evidence that it delayed flower development, caused yellowing of leaves, and increased the abscission of the reproductive structures. Temperature have several important effects on reproductive tissues, and the main phenomena observed are rapid or delayed flowering, asynchrony in the development of male and female reproductive systems (Hedhly et al., 2008), and defects in male and female gametes (Morrison and Stewart, 2002). On the other hand, a 30 °C temperature in a 16-h photoperiod was related to higher viability of pollen grains, accelerated floral development, and lower floral abscission. McLaughlin and Garofalo (2002) and Avekin (2016) also reported that *Adenium* adapts well to temperatures above +25 °C and sufficient lighting (maximum 13,000 lux), and it exhibits the best pollen fertility on the first day of anthesis.

The results also indicated a significant correlation between the development stadium of the flowers and the accessions evaluated in relation to *in vitro* pollen germination. The pollen grain germination rate was significantly higher for ICA-wd in all analyzed phases. The germination percentage was highest in pre-anthesis for accession ICA-wd, and, on the other hand, lowest, in this same phase, for accession ICA-rt. Only in accession ICA-ps pollen grain germination was uninfluenced by the phase (Figure 2).

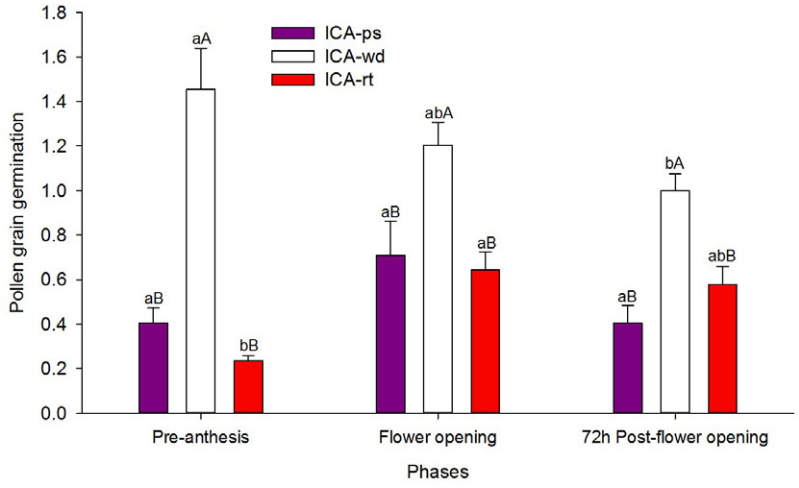

Figure 2. *In vitro* pollen grain germination of three *Adenium obesum* accessions (ICA-wd, ICA-ps, and ICA-rt) in three phases. Means have been transformed (logX + 1). Columns with the same capital letter for evaluated accessions and lowercase letter for evaluated phases not significantly different by the Tukey test at $*p < 0.05$. Bar indicates mean with standard error (SE).

The pollen viability observed was maintained for up to 3 days, the maximum period evaluated in this study (Figure 2). The results of this study for the ICA-wd accession corroborate those described by Thomson and Thomson (1989), indicating that the pollen grains tend to lose viability over time. He et al. (2017) observed the viability behavior of three *Lillium* hybrids and concluded that viability was highest at day of flower opening, and reduced over time. Therefore, pollen grains taken from older flowers are less likely to fertilize ovules than those from young flowers. The ICA-wd accession is a material with high potential for use in future hybridizations as a male progenitor since it showed higher pollen viability in general. Its pollen grains may also be used in pre-anthesis, which, in a practical way, can prevent the risk of pollen contamination for controlled hybridizations.

The accessions evaluated and the growing conditions showed that *A. obesum* plants have long high stigma receptivity (Figure 3). This finding was obtained using the peroxidase test, which is considered by several authors to be reliable for determining receptivity (Dey et al., 2016). When a species is classified as having long stigma receptivity, it may be a sign of adaptation to few visitors to increase the chances of pollination (Zhang and Wolfe, 2016). Stigma receptivity plays an important role in successful hybridization. For fertilization to occur, the pollen grains must be in a viable state, and the stigma must be in a receptive condition (Zulkarnain et al., 2019).

In the present study the stigmatic receptivity was higher at 72-h post-opening flower (Figure 3). According to some authors the stigmatic receptivity appears to be associated with the production of stigmatic secretion (Herrero and Dickinson 1981), esterase and peroxidase activities (Bernhardt et al. 1980). In desert rose, little work has been done to determine what makes a stigma unreceptive or receptive and considering our findings additional studies must be conducted in order to elucidated this condition.

A significant relationship ($p < 0.05$) between temperatures, sucrose concentrations and the characteristic of *in vitro* germination of *A. obesum* pollen grains was observed. The *in vitro* germination percentage increased with the temperature and concentration to a maximum value of 39.81%, and then decreased to a minimum value of 16.60% (Figure 4).

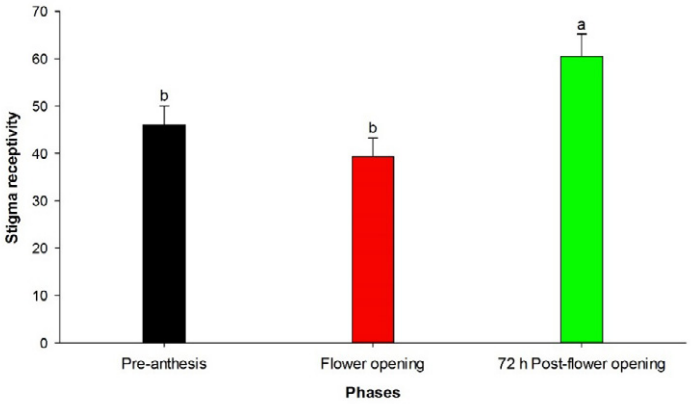

Figure 3. Stigma receptivity of accessions of *Adenium obesum* in three phases. Columns with the same lowercase letter for evaluated phases not significantly different by the Tukey test at $*p < 0.05$. Bar indicates mean with standard error (SE).

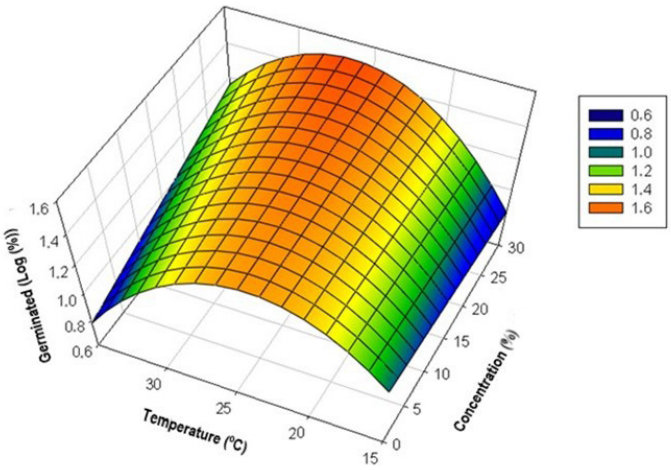

Figure 4. Response surface for *in vitro* germination of *Adenium obesum* pollen grains with respect to sucrose concentrations (0, 10, 20, and 30%) and temperatures (15, 20, 25, and 30 °C). Means have been transformed (logX + 1). $Z = -1.38065 - 0.019789**x + 0.234177**y - 0.004929**y^2 + 0.0009262**xy$. R^2 adjust. = 0.607; **Significant by *t* test at 1% probability.

The results indicated that the *in vitro* germination of pollen grains collected at *A. obesum* anthesis responded positively to the effects of temperature in the 20-30 °C range, with great variations, as for the sucrose concentration. García et al. (2012) reported that sucrose is a fundamental source of energy for pollen germination, and induces pollen tube growth and the synthesis of new cells. In this study, even in the absence of sucrose, the *in vitro* germination percentage of pollen grains was higher than 30%. This can be explained by the accession genotype, other inorganic elements (boron, calcium nitrate, magnesium sulfate and potassium nitrate) of the culture medium, as well as the use of the reserve carbohydrates present in pollen grains when they are released from anthers. The interaction with these inorganic constituent elements of this medium may have been positive to the germination of pollen grains and pollen tube growth (Souza et al. 2010).

Conclusions

Cross-fertilization is widely used to obtain new commercial variants of *A. obesum*. The association of pollen viability with stigmatic receptivity facilitates the management of artificial hybridization for the species. Considering hybridization among the accessions evaluated, it is possible to associate the moment of higher pollen viability with flowers having maximum stigma receptivity, without the need for synchronous anthesis of different accession plants. Consequently, the system becomes more efficient, avoiding losses of floral material for manipulating and conducting crosses more easily.

Author contribution

SN, CCFP, EFAA: conceived and designed research. **CGS, SMBR**: conducted experiments and investigation process. **CGS, SMBR**: collected the data. **CGS, SN, CCFP**: wrote the manuscript. **SN, EFAA, CCFP, MCTP**: provided scientific feedback and critical comments and revised the content. All authors read and approved the manuscript.

Acknowledgments

The authors would thank the Coordination for the Improvement of Higher Education Personnel (CAPES) for granting the post-graduation scholarship, and the National Council for Scientific and Technological Development (CNPq) and Minas Gerais State Agency for Research and Development (FAPEMIG) for the financial support granted for this study.

References

AVIEKIN, J.V.; GAYDARZHI, M.M Generative reproduction of *Adenium obesum* (Forssk.) Roem. & Schult. under conditions of introduction. **Plant Introduction**, v.71, p. 57-63, 2016. https://doi.org/10.5281/zenodo.2387342

BENNETT, K.; WILLIS, K.J. Pollen. In: BIRKS, H. J. B.; LAST, W. M. (eds). **Tracking Environmental Change Using Lake Sediments**. Volume 3: Indicadores terrestres, algas e siliciosos. Basingstoke: Springer, 2001. pp.5-32.

BERNHARDT, P.; KNOX, R.B.; CALDER, D.M. Floral biology and self-incompatibility in some Australian mistletoes of the genus *Amyema* (Loranthaceae). **Australian Journal of Botany**, v.28, p.437-451, 1980.

BISWAS, P. Stigma receptivity of *Corchorus aestuans* L. with reference to in vivo pollen germination and peroxidase activity. **Bioscience Discovery**, v.8, n.3, p.628-632, 2017.

BREWBAKER, J.L.; KWACK, B.H. The essential role of calcium ion in pollen germination and pollen tube growth. **American Journal of Botany**, v.50, p.859-865, 1963. https://doi.org/10.1002/j.1537-2197.1963.tb06564.x

CHOUDHARY, N.; SIDDIQUI, M.; BI, S.; KHATOON, S. Effect of seasonality and time after anthesis on the viability and longevity of *Cannabis sativapollen*. **Palynology**, v.2, p.235-241, 2014. https://doi.org/10.1080/01916122.2014.892906

COLOMBO, R.C.; CRUZ, M.A.D.; CARVALHO, D.U.D.; HOSHINO, R. T.; ALVES, G.A. C.; FARIA, R.T.D. *Adenium obesum* as a new potted flower: growth management. **Ornamental Horticulture**, v.24, p.197-205, 2018. http://dx.doi.org/10.14295/oh.v24i3.1226

COLOMBO, R.C.; FAVETTA, V.; YAMAMOTO, L.Y.; ALVES, G.A.C.; ABATI, J.; TAKAHASHI, L.S.A.; FARIA, R.T. Biometric description of fruits and seeds, germination and imbibition pattern of desert rose [*Adenium obesum* (Forssk.), Roem. & Schult.]. **Journal of Seed Science**, v.37, n.4, p.206-213, 2015. http://dx.doi.org/10.1590/2317-1545v37n4152811

DAFNI, A. **Pollination ecology**: a practical approach. New York: Oxford University Press, 1992. 250p.

DAFNI, A.; FIRMAGE, D. Pollen viability and longevity: practical, ecological, and evolutionary implications. **Plant Systematics and Evolution**, v.222, n.1-4, p.113-132, 2000. https://doi.org/10.1007/BF00984098

DEY, K., MONDAL, S., MANDAL, S. Studies on stigma receptivity of *Grewia asiatica* L. with reference to esterase and peroxidase activity. **International Journal of Engineering Research & Science**, n.2, p.120-122, 2016.

FLORES-RENTERÍA, L.; WHIPPLE, A.V.; BENALLY, G.J.; PATTERSON, A.; CANYON, B.; GEHRING, C.A. Higher temperature at lower elevation sites fails to promote acclimation or adaptation to heat stress during pollen germination. **Frontiers in Plant Science**, v.9, p.536, 2018. https://doi.org/10.3389/fpls.2018.00536

GARCÍA, C.C.; GUARNIERI, M.; PACINI, E. Tomato pollen tube development and carbohydrate fluctuations in the autotrophic phase of growth. **Acta Physiologiae Plantarum**, v.34, p.2341-2347, 2012. https://doi.org/10.1007/s11738-012-1037-4

GIOVANNINI, A.; MACOVEI, A.; CASER, M.; MANSUINO, A.; GHIONE, G.G.; SAVONA, M.; CARBONERA, D.; SCARIOT, V. pollen grain preservation and fertility in valuable commercial rose cultivars. **Alma Balestrazzi Plants**, v.6, p.17, 2017. https://doi.org/10.3390/plants6020017

HE, G.; HU, F.; MING, J.; LIU, C.; YUAN, S. Pollen viability and stigma receptivity in Lilium during anthesis. **Euphytica**, v.9, p. 213-231, 2017. https://doi.org/10.1007/s10681-017-2019-9

HEDHLY, A.; HORMAZA, J.I.; HERRERO, M. Global warming, and plant sexual reproduction. **Trends in Plant Science**, v.14, p.30-36, 2008. https://doi.org/10.1016/j.tplants.2008.11.001

HERRERO, M.; DICKINSON, H.G. Ultrastructural and physiological differences between buds and mature flowers of *Petunia* hybrid prior to and following pollination. **Planta**, v.148, 138-145, 1981. https://doi.org/ 10.1002/aps3.1038

IMPE, D.; REITZ, J.; KÖPNICK, C.; ROLLETSCHEK, H.; BÖMER, A.; SENULA, A.; NAGEL, M. Assessment of pollen viability for wheat. **Frontiers in Plant Science**, v.10, p.1588, 2020. https://doi.org/10.3389/fpls.2019.01588

KEARNS, C.A.; INOUYE, D.W. Techniques for pollination biologists. Colorado: University of Colorado Press, 1993.

KOTI, S.; REDDY, K.R.; REDDY, V.R.; KAKANI, V.G.; ZHAO, D. Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination, and tube lengths. **Journal of Experimental Botany**, v.56, p.725-736, 2005. https://doi.org/ 10.1093/jxb/eri044

MCBRIDE, K.; HENNY, R.J.; CHEN, J.; MELLICH, T.A. Effect of light intensity and nutrition level on growth and flowering of *Adenium obesum* 'Red' and 'Ice Pink'. **HortScience**, v.49, n.4, p.430-433, 2014. https://doi.org/10.21273/HORTSCI.49.4.430

MCKAIN, M.R.; MATTHEW, G.J.; SIMON, L.C.; DEREN, E.; YA,Y. Practical considerations for plant phylogenomics. **Applications in Plant Sciences,** v.6, p.1038, 2018.

MCLAUGHLIN, J.; GAROFALO, J. The Desert Rose (*Adenium obesum*). Miami-Dade: Miami-Dade County/ University of Florida Cooperative Extension Service, 2002. 66p.

MORRISON, M. J.; STEWART, D.W. Heat stress during flowering in summer Brassica. **Crop Science**, v.42, p.797-803, 2002.

MÜLLER, F.; XU, J.; KRISTENSEN, L.; WOLTERS-ARTS, M.; GROOT, P. F. M.; JASMA, S. Y.; MARIANI, C.; PARK, S.; RIEU, I. High-temperature-induced defects in tomato (*Solanum lycopersicum*) anther and pollen development are associated with reduced expression of b-class floral patterning genes. **PLoS One**, v.11, p.e0167614, 2016. https://doi.org/10.1371/journal.pone.0167614

PLAIZIER, A C. A revision of *Adenium* Roem. and Schult. and of *Diplorhynchus* Welw. *ex* Fic. and Hiern (Apocynaceae). **Mededelingen Landbouwhoge School**, v.80, p.1-40, 1980.

POSSOBOM, C.C.F.; CANAVEZE, I.; RAMOS, S.M.B. Aspectos botânicos da rosa-do-deserto. In: NIETSCHE, S.; ALMEIDA, E.F.; MENDES, R.B. (eds). **Cultivo e Manejo da Rosa-do-Deserto**. São José dos Pinhais: Brazilian Journals, 2021. p.10-39.

SOARES, T. L.; JESUS, O. N.; SOUZA, E. H.; OLIVEIRA, E. J. Reproductive biology, and pollen–pistil interactions in *Passiflora* species with ornamental potential. **Scientia Horticulturae**, v.197, p.339-349, 2015. https://doi.org/10.1016/j.scienta.2015.09.045

SOUZA, V.A.; SCHEMBERG, E.A.; AGUIAR, A.V. Germinação *in vitro* do pólen de jerivá (*Syagrus romanzoffiana* (S.) Cham). **Scientia Forestalis**, v.38, p.147-151, 2010.

THOMSON, J.D.; THOMSON, B.A. Dispersal of *Erythronium grandiflorum* pollen by bumblebees: implications for gene flow and reproductive success. **Evolution**, v.43, n.3, p.657-661, 1989. https://doi.org/10.1111/j.1558-5646.1989.tb04261.x

YI, W.; LAW, S.E.; MACCOY, D.; WETZSTTEIN, H.Y. Stigma development and receptivity in almond (*Prunus dulcis*). **Annals of Botany**, v.97, n.1, p.57-63, 2006. https://doi.org/10.1093/aob/mcj013

ZHANG, X.M. ; WOLFE, L.M.; Stigma receptivity over the lifetime of the hermaphroditic flower of *Elsholtzia rugulosa* was negatively correlated with pollen viability. **Plant Signaling & Behavior**, v.11, p.333-342, 2016. https://doi.org/ 10.1080/15592324.2016.1259052

ZULKARNAIN, Z., ELIYANTI, E., SWARI, E.I. Pollen viability and stigma receptivity in *Swainsona formosa* (G. Don) J. Thompson (Fabaceae), an ornamental legume native to Australia. **Ornamental Horticulture**, v.25, p.158-167, 2019. https://doi.org/10.14295/oh.v25i2.2011