

SCIENTIFIC ARTICLE

Morpho-anatomical and biochemical characterization of *Strelitzia reginae* seeds and embryos

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Abstract

Strelitzia or bird-of-paradise, is an important tropical ornamental plant that is difficult to propagate because of seed dormancy issues and the low number of seedlings obtained from plant divisions. With greater knowledge of its structure, it is possible to develop strategies to improve the propagation process for this species, since information on this subject is currently limited. Thus, the objective was to carry out an anatomical, morphological and biochemical characterization of *Strelitzia reginae* seeds and embryos. The characterization of the seeds was performed through image analysis using GroundEye equipment, X-rays, and anatomical and biochemical analyses. Additionally, the following biometric characteristics of the seeds were obtained: an area of 0.33 cm², a maximum diameter of 0.736 cm, a maximum lateral diameter of 0.59 cm, a minimum diameter of 0.58 cm, and a perimeter of 2.47 cm. The seeds of *S. reginae* can be considered starchy since they contain 15% starch. By integument anatomical analysis, we observed the presence of an exotesta (ex) and a posterior parenchymatic layer that was divided into a mesotest (m) and endotest (en). The characterization of *Strelitzia reginae* seeds and embryos showed relevant observations for the taxonomy and physiology of this species. The seeds are aleurostarches and present an area of 0.33 cm² on average, with maximum lateral diameter of 0.59 cm. As result of anatomical studies, it was possible to determine coat responsible for integument dormancy.

Keywords: anatomy, biochemical seed analysis, bird-of-paradise, starchy seed, *Strelitzia*, *Strelitziaceae*, tropical plant.

Resumo

Caracterização de sementes e embriões de *Strelitzia reginae*

Strelitzia ou ave-do-paráiso, é uma importante planta ornamental tropical de difícil propagação devido aos problemas de dormência das sementes e ao reduzido número de mudas obtidas nas divisões das plantas. Com o maior conhecimento de sua estrutura, é possível desenvolver estratégias para melhorar o processo de propagação dessa espécie, uma vez que as informações sobre estes aspectos são limitadas. Assim, o objetivo foi caracterizar sementes e embriões de *Strelitzia reginae*. A caracterização das sementes foi realizada por meio de análise de imagens em equipamento GroundEye, Raio-X e análises anatômicas e bioquímicas. Adicionalmente, foram obtidas as seguintes características biométricas das sementes: área de 0,33 cm², diâmetro máximo de 0,736 cm, diâmetro lateral máximo de 0,59 cm, diâmetro mínimo de 0,58 cm e perímetro de 2,47 cm. As sementes de *S. reginae* podem ser consideradas amiláceas por conterem 15% de amido. Pela análise anatômica do tegumento, observou-se a presença de uma exotesta (ex) e uma camada parenquimática posterior que foi dividida em mesotesta (m) e endotesta (en). A caracterização de sementes e embriões de *Strelitzia reginae* apresentou observações relevantes para a taxonomia e fisiologia desta espécie. As sementes são aleuroamiláceas e apresentam área média de 0,33 cm², com diâmetro lateral máximo de 0,59 cm. Como resultado de estudos anatômicos, foi possível identificar o tegumento responsável pela dormência.

Palavras-chave: anatomia, análise bioquímica de sementes, planta tropical, semente amilácea, *Strelitzia reginae*, *Strelitziaceae*

Introduction

The main species of tropical flowers belong to the order Zingiberales, which naturally occur in tropical areas of the Americas, West Asia, Africa, and the Pacific. Zingiberales

are herbaceous, rhizomatous, perennial shrubs characterized by colored bracts and varied shapes. Commercially, they present high postharvest durability and beauty and are recommended for enhancing the beauty of environments and gardens (Paiva et al. 2012; Vieira et al., 2021). The

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<https://doi.org/10.1590/2447-536X.v28i3.2456>

Received: Nov 25, 2021 | Accepted: May 4, 2022 | Available online: June 22, 2022

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Area Editor: Carmen Silvia Zickel

genus *Strelitzia* belongs to the order Zingiberales and family Strelitziaceae (Cron et al., 2012).

The Strelitziaceae family consists of three genera: *Phekanospermum*, *Ravenala* and *Strelitzia* (Oliveira et al., 2012). The genus *Strelitzia* has five species, all with ornamental value: *S. reginae* Banks ex Aiton, *S. juncea*, *S. nicolai*, *S. alba*, and *S. caudata*, all originating from southern Africa (Cron et al., 2012). Among these, the most commercially used species, *Strelitzia reginae*, comes from areas along riverbanks in coastal Bush or from coastal Bush, mainly in the Eastern Cape (Cron et al., 2012).

Strelitzia reginae Banks ex Aiton is also popularly known as the bird-of-paradise, queen-of-paradise, toucan's beak, flower-of-paradise, queen flower or garden banana (Vieira, 2012). *Strelitzia* has great commercial value, with attractive colorful inflorescences, and is widely used for both the production of cut flowers and seedlings for gardens (Paiva et al., 2012; Vieira et al., 2014).

Propagation can be carried out by seeds or by dividing clumps, which results in a low number of seedlings. Due to dormancy, the germination process and seedling development are slow, and flower production only begins after 5 to 6 years of cultivation (Paiva et al., 2012). For seed production, flowers can be pollinated naturally by insects such as ants and wasps, in addition to birds, which are attracted by the hexose-dominant nectar (Kronstedt and Walles, 1986). Additionally, a manual process can be used, providing an increase in the number of seeds/fruits (Paiva et al., 2012). The time between pollination and seed harvest may vary from 5 to 6 months (Criley, 1988). Seeds are formed in woody capsules and feature bright orange arils. This color is associated with the presence of bilirubin, a rare substance in plants (Pirone et al., 2010; Dwarka et al., 2020). Apparently, the aril does not have a specific function and must be removed before disposing of the seeds for germination (Criley, 1988), although this structure can serve as an attractant for birds, helping for its dispersal.

However, the use of conventional propagation methods is extremely slow, limiting seedling production (Paiva et al., 2007, 2012). Therefore, to develop an alternative, some studies for propagation through tissue culture have been conducted, which were successful when embryos or seeds were used as explants. However, there is still a limitation for this technique since there is no protocol for *in vitro* multiplication or production of higher shoot number, although some studies have investigated these processes (Paiva et al., 2004; Paiva et al., 2007; Figueiredo et al., 2021a).

Considering the occurrence of dormancy in the seeds, chemical scarification using concentrated sulfuric acid during 9 min (Barbosa et al., 2005) or HCl – 1 mL L⁻¹ for 1 min (Kumar et al., 2018) or physical scarification through sandpaper are suggested (Carvalho et al., 2020), although these processes of disrupting dormancy have not yet been fully elucidated.

Overall, information on this species is still limited, and the main available studies focus on postharvest conservation, floral development (Kronstedt and Walles, 1986), phylogeny (Cron et al., 2012), and *in*

vitro propagation (Paiva et al., 2000; Paiva et al., 2004; Paiva et al., 2007; Figueiredo et al., 2021a; 2021b). Some studies have already analyzed the evolution, species and morphological characteristics of the plant. Considering the characterization of seeds, there are some results for the genus *Phenakospermum* and some initial studies for *Strelitzia reginae* (Benedict et al., 2016). These genera were identified as having a seed that is striate with an elongated embryo and a hair-like orange aril; it lacks an operculum. The seed coat presents isodiametric exotesta, mesotesta of one cell type, and elongated sclerenchymatous endotesta, and these characteristics are considered typical of the *Strelitziaceae* family (Benedict et al., 2016). Seed characterization information would be essential to elucidate the germination process *in vitro* and *ex vitro*, helping studies identify greater efficiency for these processes.

However, there are no comprehensive characterizations of *Strelitzia* seeds. Sane et al. (2020) carried out studies with several accessions, verifying the phenotypic characteristics of the aerial part of the plant and the inflorescences, but they did not carry out a characterization of the seeds.

Thus, in this context, the objective was to carry out an anatomical, morphological and biochemical analysis for a better understanding of *S. reginae* seeds and embryos characteristics.

Material and Methods

For characterizing *Strelitzia reginae*, seeds were obtained from dried fruits collected from plants grown under the full sun. Three replicates with ten seeds each were used, which were placed in an acrylic tray in a GroundEye® device for analysis. The software was calibrated, and images of both sides of the seeds were captured. Through these images, the color of the seeds and the geometric aspects of the seeds, such as their area (cm²), diameter (cm), thinning, perimeter (cm), circularity, and contour (cm), were analyzed.

Internal morphological characterization of *Strelitzia reginae* seeds

The internal morphology of the seeds was characterized using X-ray images. The seeds were placed in trays, keeping the embryonic axis region on the top of the cells. For that, 30 seeds were used, with 3 repetitions of 10 seeds each. The radiographic images of the seeds were exposed to intensities of 25, 30, 35, and 50 kV for 30, 45, 60 and 90 seconds of exposure. The X-ray machine used was a Faxitron HP®, Model 43855AX, with Kodak Min-R 2000 radiographic film, dimensions 18 x 24 cm. The X-ray processing was performed through a Kodak brand X-ray processing processor, Model M35X OMAT. According to the internal morphology visualized on the radiographs, the seeds were categorized based on viability.

Anatomical evaluations of *Strelitzia reginae* seeds

The seeds were manually cut into cross-sections and then stained using safranin and Astra blue for anatomical evaluations, histological analysis of the integuments, and determination of the type of reserve. The seeds were

also stained with Lugol to identify the presence of starch (Kraus and Arduin, 1997). The assembly of temporary and semipermanent cutting blades was performed according to the techniques described by Johansen (1940). Visualizations were performed using a Zeiss® microscope, with photographic documentation using a Canon® digital camera with a resolution of 8.0 megapixels and 8x optical zoom.

Biochemical analyses of *Strelitzia reginae* seeds

For biochemical analysis, 30 seeds, 3 replications of 10 seeds each, were dehydrated in a forced circulation oven at 70 °C until they reached constant weight. After this procedure, the seeds were macerated and then subjected to an extraction process. The humidity content and the ether extract of the seeds were determined according to the AOAC method – Official Methods of Analysis (2019). The protein fraction was obtained by determining the percentage of total nitrogen in the sample according to the Kjeldahl method (AOAC, 2019) and multiplying by a factor of 6.25. The crude fiber was determined by the gravimetric method of Kamer and Ginkel (1952). Fixed mineral waste (ash) was determined according to the AOAC method (2019) by incinerating the material in a muffle oven set at 550 °C. The starch content, total soluble sugars, and reducing and non-reducing sugars were obtained according to the Somogy method, adapted from Nelson (1944).

Strelitzia reginae seed soaking curve

The *Strelitzia reginae* seeds was placed on Germitest® paper moistened with distilled water equivalent to 2.5 times the weight of the paper (25 °C ± 2 °C) and then kept in a B.O.D. under controlled conditions (photoperiod of 16 hours, temperature of 25 ± 2 °C and photon irradiance of 36 µmol m⁻² s⁻¹). Four replicates with 10 seeds each were used.

To determine the imbibition curve for the seeds of *Strelitzia reginae*, the seeds were weighed for 408 h; the first weighing was carried out on the dry seeds and then they were weighed every 30 minutes for the first 12 hours. After this period, data were collected at increasing intervals of 1 h, 3 h, 4 h, 6 h, and, finally, every 12 hours until the end of the evaluations.

The germination test for the *Strelitzia reginae* seeds considered root protrusion at ± 2.0 mm as the criterion for determining germination. At the end of 408 hours (17 days), the seeds were removed from the Germitest® paper rolls, weighed again (final mass), and then placed in an oven at 105 ± 2 °C for 24 hours to determine moisture content (%).

Statistical analyses

Analyses morphological and biochemical were conducted in a completely randomized design (CRD) with 3 replications of 10 seeds each. Using the data obtained, the general average of each analyzed parameter was determined.

For anatomical analyses, descriptive statistic was used, based on the photographs taken, anatomical description and simple averages for some.

For seed soaking curve, the analysis was also conducted in a completely randomized design (CRD) with 4 replications of 10 seeds each. After the period of analysis, the general average of each time was determined. Using these data, a graphic was created, and the best curve was defined.

Results and Discussion

The analysis performed allowed us to determine *Strelitzia reginae* seed coloration. The images showed that the black color was predominant, occurring in 80% of the seeds, but smaller amounts of blue (7%), sky blue (7%), and dark gray (6%) were also present (Figure 1).

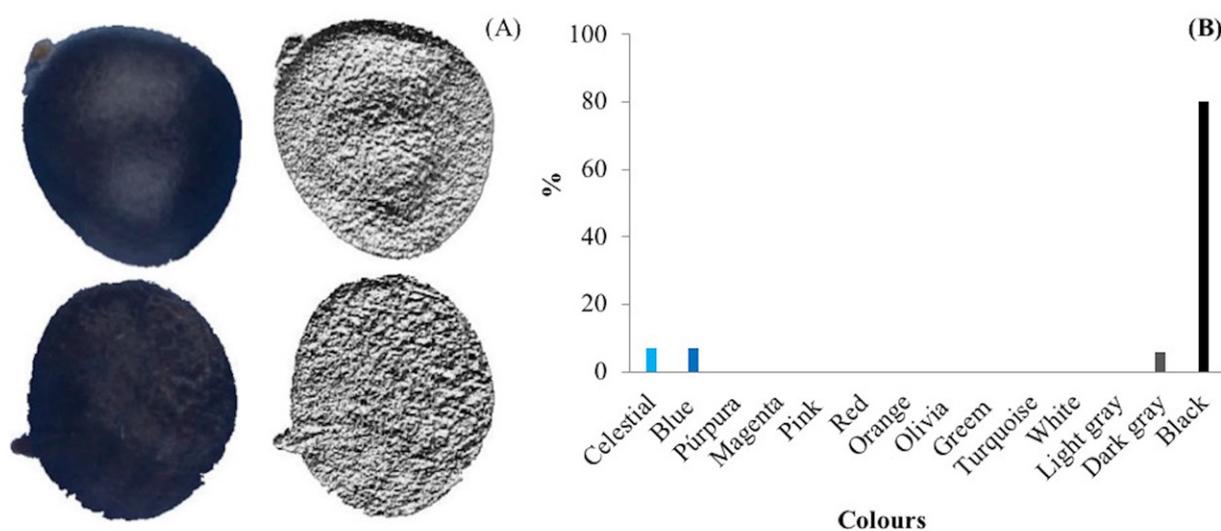


Figure 1. GroundEye® image analysis of *Strelitzia reginae* seeds (A) and color predominance in *Strelitzia reginae* seeds (B).

Although the seeds of *Strelitzia reginae* (Pirone et al., 2010; Paiva et al., 2012), *Strelitzia nicolai* (Duretto et al., 2017), and *Phenakospermum guyannense* (Oliveira et al.; 2012) were described as only black in color, the analysis performed indicated the existence of other colors, such as blue and gray. Notably, *in vitro* oxidation compounds on a medium culture during *in vitro* cultivation were identified, and different colors were present in the exudates, including blue (Paiva et al., 2000).

The average parameters of the morphometric characteristics of the *Strelitzia* seeds were determined by analysis using GroundEye®. The average size of the seeds (area) and the space occupied by them (convex area) were measured. The area of the seeds was 0.33 cm² on average, and the convex area was 0.34 cm². The *Strelitzia reginae* seeds had an average maximum lateral diameter of 0.59 cm and an average minimum lateral diameter of 0.58 cm. Contour irregularity was determined in the *Strelitzia reginae* seeds, with an average of 0.07, and the seed perimeter and convex perimeter were 2.42 and 2.09 cm, respectively (Table 1).

Table 1. Morphometric characteristics of *Strelitzia reginae* seeds determined by GroundEye® analysis.

Morphometric characteristics	Average	Standard deviation
Area	0.33 cm ²	5.61 mm ²
Circumference	0.78	0.07
Spherical shape	18.07	4.14
Convex area	0.34 cm ²	5.65 mm ²
Contained diameter	0.55 cm	0.50 mm
Maximum diameter	0.74 cm	0.81 mm
Maximum lateral diameter	0.59 cm	0.55 mm
Minimum lateral diameter	0.58 cm	0.52 mm
Contour irregularity	0.07	0.04
Number of gaps	0.00	0.00
Perimeter	2.42 cm	0.26 cm
Convex perimeter	2.09 cm	0.18 cm

Although different studies have provided morphoanatomical descriptions of species belonging to the Strelitziaceae family (Oliveira et al.; 2012) and some have described the morphological aspects of the seeds of the genus *Strelitzia* (Duretto et al., 2017), there is no characterization of the complete morphometric analysis of *Strelitzia reginae* seeds.

For *Strelitzia nicolai*, it was determined that the seed was between 8-10 mm in diameter (Duretto et al., 2017), thus indicating that these seeds were smaller than those of *Strelitzia reginae*, which had diameters of 5.5 mm (contained diameter) and 7.4 mm (maximum diameter). The contained diameter is the largest diameter of the circumference that fits inside the object (seed) (Acha and Vieira, 2020). For contour irregularity, which defines the object's adjustment level (Acha and Vieira, 2020), the seeds presented 0.7. No gaps were detected in the seeds. Duretto et al. (2017) classified *Strelitzia reginae* seeds as small and globose with an orange aryl.

Seeds of *Phenakospermum guyannense*, a species of the Strelitziaceae family, were characterized as oval seeds with

an average length of 9.74 mm, the width of 6.42 mm, and thickness of 5.86 mm (Oliveira et al., 2012), showing to be an oval seed in comparison with *S. reginae* seeds, which were already classified as globular seeds (Duretto et al., 2017), now confirmed by the present work.

Different studies have carried out morphological descriptions of arils (Serrato-Valenti et al., 1991) due to their peculiar color and promising compounds for commercial use, but there are few studies on the characterization of *Strelitzia reginae* seeds (Duretto et al. 2017).

Characterization of the internal morphology of *S. reginae* seeds

No internally damaged or empty seeds were detected by radiographic analysis, which showed that the seeds were completely full internally. Through an X-ray test, it is possible to analyze the internal morphology of the seed, allowing the selection of viable and nonviable seeds (Medeiros et al., 2020). In this way, it was demonstrated that all the analyzed *Strelitzia reginae* seeds presented viable characteristics based on internal morphology (Figure 2).

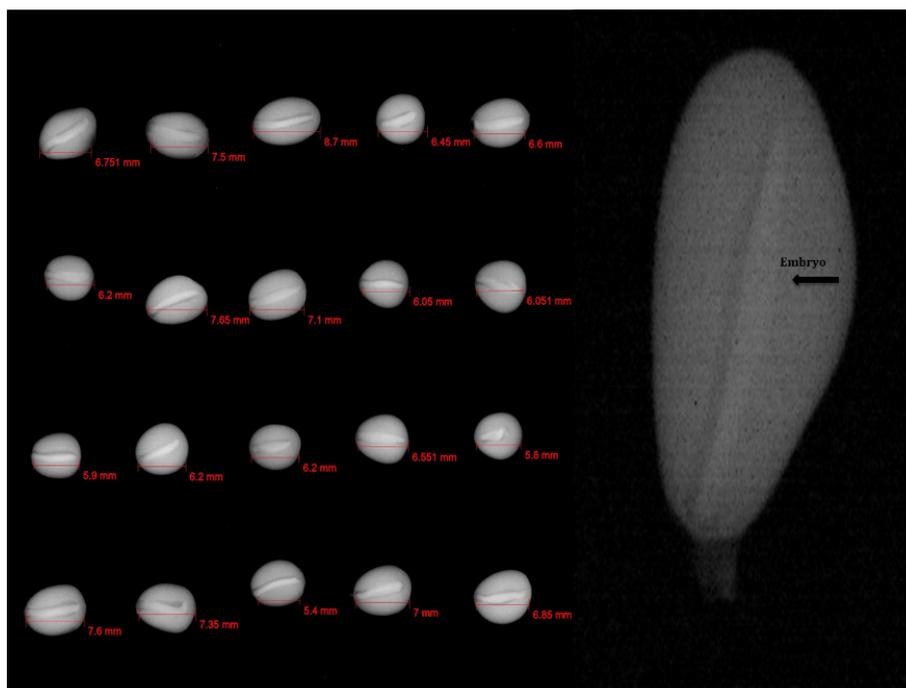


Figure 2. Analysis of X-ray images of *Strelitzia reginae* seeds.

In the images, it is possible to observe that the embryo occupies the seed in a linear direction over the entire area. The absence of cotyledons is also not observed. Interestingly, embryos, regardless of the size of the seeds, tend to occupy the entire dimensions.

However, due to natural variation, some seeds, even without apparent damage in the X-ray tests, may not germinate because of infections by microorganisms or physiological damage, but it is not possible to demonstrate the practical viability of the seeds using this analysis. Another factor affecting viability would be tegumentary and/or chemical

dormancy that does not involve damage to the embryo (Paiva et al., 2004; Figueiredo et al. 2021a, 2021b), since uniform seed germination can be inhibited by structures that promote resistance to water imbibition (Marcos Filho, 2005).

Anatomical evaluations of *Strelitzia reginae* seeds

Through anatomical studies, it was possible to determine the aspects of the seed coat responsible for integument dormancy (physical dormancy) and to verify the presence of the lucid line (LI) that is very evident along with the integument extension (Figure 3).

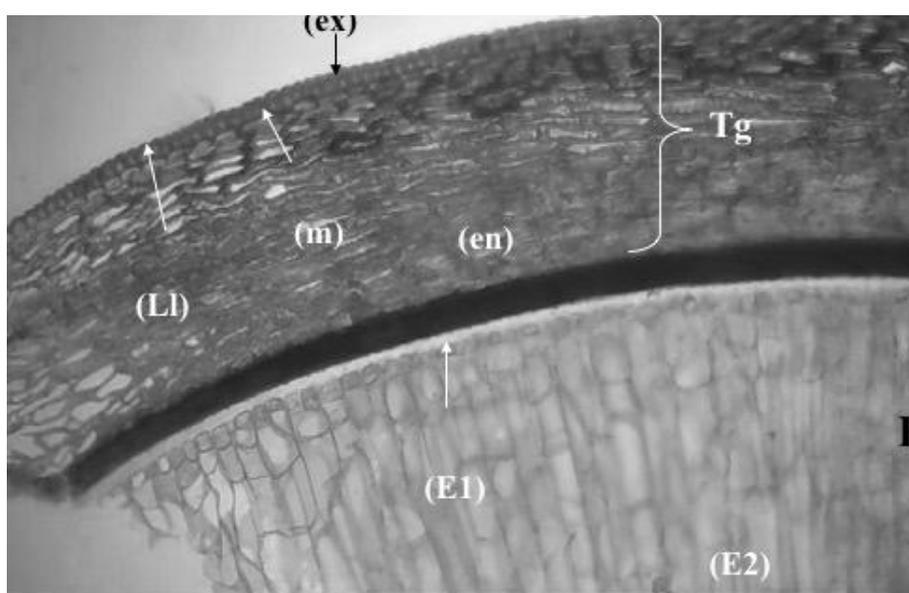


Figure 3. Anatomical aspects of a *Strelitzia reginae* seed. Integument (Tg), endosperm (End, E 1 and E 2), lucid line (LI), exotest (ex), mesotest (m) and endotest (en).

In the anatomical analysis of the integument, we observed the presence of a first isodiametric exotesta (ex), the outermost layer of cells with unistratified layers composed of tetrahedral cells with thickened walls and a posterior parenchymatic layer arranged in a compact manner and consisting of cell walls of uneven thickness. This parenchymal layer can be divided into a mesotest (m) with one cell type and an endotest (en) with elongated sclerenchymal cells (Benedict et al., 2016).

In studies with *Phenakospermum guyanense*, the formation of layers in the seed coat was also observed, in which the presence of phenolic compounds in the outermost layer of the seed was observed (Oliveira et al., 2012).

Typically, Zingiberales seeds have a perisperm of nuclear origin adjacent to the envelope and a micropylar region (Liao and Wu, 2000). The micropylar region in *Strelitzia reginae* is formed by the aryl, the endosperm, and the embryos, and the chalazal region is formed by the embryo, the endosperm and endotestal chalazal thickening (Benedict et al., 2016).

In *P. guyanense* seeds, the formation of a perisperm can be observed, but the micropyle region was not found (Oliveira et al., 2012). The absence of the micropylar region in *Strelitzia reginae* and *Phenakospermum guyanense* is justified, as this region is not found in basal Zingiberales families, such as Strelitziaceae and Heliconiaceae (Grootjen and Bouman, 1981; Graven et al., 1996).

The parenchyma layer is considered responsible for the impermeability of the hardest seeds. This impermeability occurs due to the physical arrangement of this palisade

layer and chemical coatings impregnated in its cells (lignin, cutin, suberin, waxes) that prevent the absorption of water (Baskin et al., 2000) and the lucid line (L I), which occurs throughout the integument (Baskin et al., 2000). The lucid line is an optical phenomenon generated by the juxtaposition of inner cells in a cellulosic palisade with suberized outer cells (Kelly et al., 1992).

The endosperm (END) presents a solid, homogeneous feature over a large proportion of the seed (Figure 3). The outermost layer of the endosperm (E 1) is formed by a single stratum of cells, different from the others in that they present in tiny and compact tetrahedral shapes (Figure 3, E1). The other layers of the endosperm are composed of isodiametric cells (Figure 3, E2) that are voluminous with thin walls, probably composed of starch (Table 2).

The formation of thin-walled cells in the endosperm was also observed in *P. guyanense* seeds, in contrast to other monocotyledonous species that normally present thick-walled (Oliveira et al., 2012).

Biochemical analysis of *Strelitzia reginae* seeds

The mature seeds of *Strelitzia reginae* had approximately 48.73% humidity. The seeds of *S. reginae* can be considered aleurostarches because of their high quantities of starch (15.77 g 100 g⁻¹) and their high concentrations of proteins (10.24%) (Marcos Filho, 2005). In addition to these values, the fiber (13.55%), ash (2.49%), ether extract (1.18%), total soluble sugars (0.29 g 100 g⁻¹), sucrose (0.21 g/100 g), and reducing sugar (0.10 g 100 g⁻¹) contents are shown in Table 2.

Table 2. Chemical composition of *Strelitzia reginae* seeds.

Components	Averages (%)
Ethereal extract (%)	1.18
Proteins (%)	10.24
Reducing sugars g 100 g ⁻¹	0.10
Sucrose g 100 g ⁻¹	0.21
Starch g 100 g ⁻¹	15.77
Total soluble sugars g 100 g ⁻¹	0.29
Fibers (%)	13.55
Ashes (%)	2.49
Water content (%)	48.73

The function of starch is exclusive to the reserve, and it is also the carbohydrate most commonly found in seeds. Thus, starch seeds have greater storage potential than oilseeds due to the greater chemical stability of starch compared with lipids (Marcos Filho, 2005), which may explain the durability of the seeds after harvest. However, the abundant presence of proteins and the occurrence of lipids (ethereal extract) can contribute to the reduction in storage time due to the chemical instability of the lipids and the high affinity of proteins for water (Oliveira et al., 2012).

Strelitzia reginae seeds had higher crude fiber than

sesame (11.9%) and quinoa (7.63%), species that are popularly known as fiber sources (Câmara et al., 2020). Thus, we can conclude that *Strelitzia reginae* seeds are rich in fiber, but we cannot consider these fibers to be nutritious without specific studies

Strelitzia reginae seed soaking curve

Strelitzia reginae seeds did not present the three stages of imbibition homogeneity, and during the time of analysis, it was not possible to reach 60% germination at the end of the evaluations (Figure 4). Soon after germination, the seedlings showed radicle oxidation.

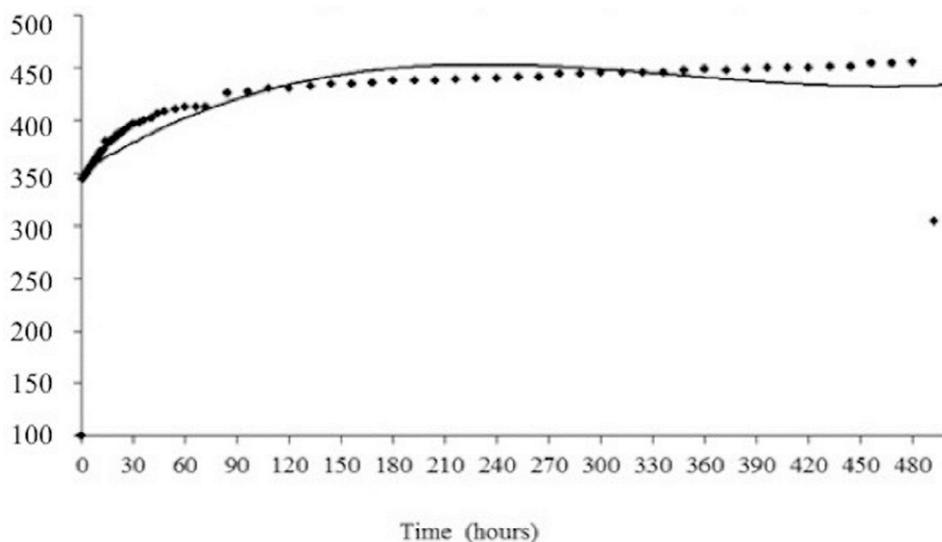


Figure 4. *Strelitzia reginae* seed imbibition curve, demonstrating the increase in initial mass (%) in relation to imbibition time.

In a period of 408 hours, the germinating seeds were weighed, and the germination percentage of the seeds of *S. reginae* was 30%, which demonstrates the need for a method of breaking integumentary dormancy as there was potentially no water absorption by the remaining seeds; this response may be related to the hard and dense endocarp. Therefore, it is believed that *Strelitzia reginae* seeds have tegumentary dormancy, since the integumentary barrier is physical, preventing the necessary imbibition for germination to occur. Thus, germination becomes limited, caused by the impermeability of the seed tissues, imposing a mechanical barrier that culminates in a low germination rate or a delay in the process.

Conclusions

The characterization of *Strelitzia reginae* seeds and embryos showed relevant observations for the taxonomy and physiology of the species. The morphometric aspects measured as well as the image analysis determined in the study characterized the seeds and the embryos of *Strelitzia reginae*. The quantification of constituents and nutritive reserve demonstrated that the seeds of *Strelitzia reginae* were aleurostarches, that is, they present a high concentration of starch and protein.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The authors would also like to thank the Fundação de Amparo à Pesquisa de Minas Gerais - FAPEMIG and National Council for Scientific and Technological Development - CNPq for the scholarships, grant (CNPQ 305066-2017-0) and the financial support provided by the project CNPQ 421765-2018-6.

Author contribution

MT: Data curation, Investigation, Methodology Writing - original draft. **PDOP:** Conceptualization, Methodology, Supervision, Funding acquisition, Writing - review and editing. **DPCS:** Conceptualization, Investigation, Data curation, Writing - original draft. **FCN:** Data curation, Methodology, Supervision, Writing - original draft. **RP:** Methodology, Funding acquisition, Writing - review and editing. **MVR:** Conceptualization, Methodology, Supervision, Writing - review and editing.

References

- ACHA, A.J.; VIEIRA, H.D. Digital image processing of coated perennial-soybean seeds and correlation with physiological attributes. **Journal of Seed Science**, v.42, e202042004, 2020. <http://dx.doi.org/10.1590/2317-1545v42227516>
- AOAC - Association of Official Analytical Chemists. Official Methods of Analysis, 21ed. AOAC International, Rockville. 2019.
- BARBOSA, J.G.; ALVARENGA, E.M.; DIAS, D.C.F.S.; VIEIRA, A.N. Efeito da escarificação ácida e de diferentes temperaturas na qualidade fisiológica de sementes de *Strelitzia reginae*. **Revista Brasileira de Sementes**, v.27, n.1, p.71-77, 2005. <https://doi.org/10.1590/S0101-31222005000100009>
- BASKIN, J.M.; BASKIN, C.C.; LI, X. Taxonomy, anatomy and evolution of physical dormancy in seeds. **Plant Species Biology**, v.15, p.139-152, 2000. <https://doi.org/10.1046/j.1442-1984.2000.00034.x>

- BENEDICT, J.C.; SMITH, S.Y.; SPECHT, C.D.; COLLINSON, M.E.; LEONG-SKORNICKOVA, J.; PARKINSON, D.Y.; MARONE, F. Species diversity driven by morphological and ecological disparity: a case study of comparative seed morphology and anatomy across a large monocot order. **AoB Plants**, v.8, plw063, 2016. <https://doi.org/10.1093/aobpla/plw063>
- CÂMARA, A., MARQUES, B.L.M.; LIRA, K.H.D.S.; SOUSA JÚNIOR, F.C.; PASSOS, T.S.; ASSIS, C.F. Study of the chemical composition of *Chenopodium quinoa* seed. **Brazilian Journal of Development**, v.6, n.6, p.34209-34226, 2020. <https://doi.org/10.34117/bjdv6n6-099>
- CARVALHO, F.J.; CARVALHO, M.; CUNHA, J.A. Dormancy breaking methods for bird of paradise seeds. **Revista Magistra**, v.31, p.799-804, 2020.
- CRILEY, R.A. Propagation of tropical cut flowers: *Strelitzia*, *Alpinia* and *Heliconia*. **Acta Horticulturae**, n.226, p.509-517, 1988.
- CRON, G.V.; PIRONE, C. BARTLETT, M.; KRESS, W.J.; SPECHT, C. Phylogenetic relationships and evolution in the *Strelitziaceae* (Zingiberales). **Systematic Botany**, v.37, n.3, p.606-619, 2012.
- DURETTO, M.F.; MCCUNE, S.; LUXTON, R.; MILNE, D. *Strelitzia nicolai* (Strelitziaceae): a new species, genus and family weed record for New South Wales. **Telopea**, v.20, p.1-3, 2017. <https://doi.org/10.7751/telepea11022>
- DWARKA, D.; MURUGAN, N.; THAYER, V.; BAIJNATH, H. A comparative microstructural study of the animal pigment, bilirubin, in seed arils of *Strelitzia nicolai*. **South African Journal of Botany**, v.128, p.109-118, 2020. <https://doi.org/10.1016/j.sajb.2019.09.016>
- FIGUEIREDO, J.R.M.; PAIVA, P.D.O.; SILVA, D.P.C.; PAIVA, R.; MESQUITA, R.; SOUZA, R.R.; REIS, M.V. Anatomical changes and cytogenetic stability in bird of paradise plants after zygotic embryo cryopreservation by desiccation method. **In Vitro Cell Development and Biology - Plant**, v.54, p.272-280, 2021b. <https://doi.org/10.1007/s11627-020-10149-x>
- FIGUEIREDO, J.R.M.; PAIVA, P.D.O.; SILVA, D.P.C.; PAIVA, R.; SOUZA, R.R.; REIS, M.V. Temperature and GA₃ on ROS and cytogenetic stability during *in vitro* cultivation of *strelitzia* zygotic embryos. **Ciência e Agrotecnologia**, v.45, e020220, 2021a. <https://doi.org/10.1590/1413-7054202145020220>
- GRAVEN, P.; KOSTER, C.G.; BOON, J.J.; BOUMAN, F. Structure and macromolecular composition of the seed coat of the Musaceae. **Annals of Botany**, v.77, p.105-122, 1996.
- GROOTJEN, C.J.; BOUMAN, F. Seed structure in *Cannaceae*: taxonomic and ecological implications. **Annals of Botany**, v.61, p.363-371, 1981.
- JOHANSEN, D.A. **Plant Microtechnique**. New York and London: McGraw-Hill Book Co. Ltd. 1940. 523p.
- KAMER, S.B.; GINKEL, L. Rapid determination of crude fiber in cereals. **Cereal Chemistry**, v.19, n.4, p.239-251, 1952.
- KELLY, K.M.; VAN STADEN, J.; BELL, W.E. Seed coat structure and dormancy. **Plant Growth Regulators**, v.11, p.201-209, 1992.
- KRAUS, J.E.; ARDUIN, M. **Manual básico de métodos em morfologia vegetal**. Rio de Janeiro: EDUR, 1997. 198p.
- KRONESTEDT, E.; WALLEES, B. Anatomy of the *Strelitzia reginae* flower (Strelitziaceae). **Nordic Journal of Botany**, v.6, p.307-320, 1986. <https://doi.org/10.1111/j.1756-1051.1986.tb00884.x>
- KUMAR, S.S.; SANE, A.; REDDY, M.L.; SAHIJRAM, L.; RAO, A.V.D.D. Effect of growth regulators on seed germination in bird of paradise (*Strelitzia reginae* L.). **Bulletin of Environmental Pharmacology - Life Science**, v.7, (Spl 1), p.101-104, 2018.
- LIAO, J.P.; WU, Q.G. A preliminary study of the seed anatomy of Zingiberaceae. **Botanical Journal of the Linnean Society**, v.134, p.287-300. 2000.
- MARCOS FILHO, J. **Fisiologia de Sementes de Plantas Cultivadas**. Piracicaba: FEALQ. 2005. 495p.
- MEDEIROS, A.D.D.; SILVA, L.J.D.; RIBEIRO, J.P.O.; FERREIRA, K.C.; ROSAS, J.T.F.; SANTOS, A.A.; SILVA, C.B.D. Machine learning for seed quality classification: An advanced approach using merger data from FT-NIR Spectroscopy and X-ray Imaging. **Sensors**, v.20, p.4319, 2020. <https://doi.org/10.3390/s20154319>
- NELSON, N. A photometric adaptation of the Somogyi method for the determination of glucose. **Journal of Biological Chemistry**, v.153, n.1, p.375-384, 1944. [https://doi.org/10.1016/S0021-9258\(18\)71980-7](https://doi.org/10.1016/S0021-9258(18)71980-7)
- OLIVEIRA, A.B.; CASSINO, M.F.; GURGEL, E.S.C.; SOUZA, M.A.D.; CARMELLO-GUERREIRO, S.A.; MEIRA, R.M.S.A.; MENDONÇA, M.S. Morfoanatomia e histoquímica da semente de sororoca (*Phenakospermum guyanense* (Rich.) Endl. - Strelitziaceae). **Revista Brasileira de Sementes**, v.34, n.2, p.280-287. 2012. <https://doi.org/10.1590/S0101-31222012000200013>

- PAIVA, P.D.O.; CARDOSO, M.G.; PASQUAL, M.; PAIVA, R. Identificação de compostos liberados no meio de cultura pelo processo de oxidação em cultivo *in vitro* de estrelícia. **Ciência e Agrotecnologia**, v.25, n.5, 2000.
- PAIVA, P.D.O.; LANDGRAF, P.R.C.; PIVETTA, K.F.L. Estrelícia. In: PAIVA, P.D.O., ALMEIDA, E.F.A. **Produção de flores de corte**. Lavras: Editora UFLA, 2012. 311-328 pp.
- PAIVA, P.D.O.; PAIVA, R.; PASQUAL, M.; PAIVA, L.V. Estabelecimento *in vitro* de estrelícia (*Strelitzia reginae* Banks.). **Ciência e Agrotecnologia**, v.28, n.5, p.1031-1037, 2004. <https://doi.org/10.1590/S1413-70542004000500009>
- PAIVA, P.D.O.; PASQUAL, M.; PAIVA, R., Controle de oxidação no cultivo *in vitro* de embriões de estrelícia (*Strelitzia reginae*). **Revista Brasileira de Horticultura Ornamental**, v.13, p.107-112, 2007. <https://doi.org/10.14295/rbho.v13i2.213>
- PANZA, V.; PIGHIN, D.; LÁINEZ, V.; POLLERO, R.J.; MALDONADO, S. Storage lipids and proteins of *Euterpe edulis* seeds. **Biocell**, v.33, n.2, p.99-106, 2009. <http://www.scielo.org.ar/pdf/biocell/v33n2/v33n2a04.pdf>
- PIRONE, C.; JOHNSON, J.V.; QUIRKE, J.M.E.; PRIESTAP, H.A.; LEE, D. The animal pigment Bilirubin is identified in *Strelitzia reginae*, the bird of paradise flower. **HortScience**, v.45, n.9, p.1411-1415, 2010. <https://doi.org/10.21273/hortsci.45.9.1411>
- SANE, A.; SUJATHA, S.; SHILPA, K.N.; LAXMAN, R.H.; SHIVASHANKARA, K.S. Growth, yield, physiological and biochemical traits of different accessions of bird of paradise (*Strelitzia reginae* L.). **Industrial Crops Production**, v.151, e112477, 2020. <https://doi.org/10.1016/j.indcrop.2020.112477>
- SERRATO-VALENTI, G.; CORNARA, L.; MODENESI, P.; PROFUMO, P. The aril of the *Strelitzia reginae* Banks Seed: structure and histochemistry. **Annals of Botany**, v.67, n.6, p.475-478, 1991. <https://doi.org/10.1093/oxfordjournals.aob.a088187>
- VIEIRA M.R.S.; SIMÕES A.N.; SOUZA P.A. Recommended temperature and relative humidity for storage of Brazilian tropical flowers. **African Journal Biotechnology**, v.13, n.11, p.1198-1201, 2014.
- VIEIRA, M.R.S. Genus: *Strelitzia*. **Journal of Horticulture and Forestry**, v.4, n.11, p.178-180, 2012.
- VIEIRA, M.R.S.; LIMA, G.P.P.; MEDEIROS, D.C.; SOUZA, A.V.; OLIVEIRA, E.C.A. The economic importance of *Strelitzia* in floriculture. **International Journal of Horticulture and Floriculture**, v.9, n.1, p.1-3, 2021.