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Dormancy breaking methods for bird of paradise seeds

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Abstract: The bird of paradise is one of the most commercialized flowers in Brazil. The biggest difficulty in its seed propagation is the low germination percentage that occurs due to the physiological seed dormancy. It is necessary to study methods that can enable the commercial seedlings production from seeds, seeking efficient treatments to overcome dormancy. The aim of this research was the find the best treatment to break bird of paradise's seed dormancy. It was tested methods of scarification with or without the addition of gibberellin, totaling six dormancy break treatments. The highest germination percentage for bird of paradise seeds is obtained using scarification with sandpaper in the micropyle region with 24 hours of imbibition with gibberellin.

Keywords: Physiological dormancy; *Strelitzia reginae* L., Gibberellin.

Métodos de quebra de dormência em sementes de ave-do-paraíso

Resumo: A ave-do-paraíso é uma das flores mais comercializadas no Brasil. A maior dificuldade em sua propagação por sementes é dada pela baixa germinação que ocorre devido à dormência fisiológica. É necessário o estudo de métodos que permitam a produção de mudas comerciais a partir de sementes, procurando-se tratamentos para sua quebra de dormência. O objetivo da pesquisa foi encontrar o melhor método para quebra de dormência de sementes de ave-do-paraíso. Foram testados métodos de quebra de dormência por escarificação, com ou sem adição de giberelina, totalizando seis tratamentos A maior taxa de germinação em sementes de ave-do-paraíso é obtida utilizando a escarificação mecânica na região da micrópila juntamente com a embebição em giberelina por um período de 24 horas.

Palavras chave: Dormência fisiológica, *Strelitzia reginae* L., Giberelina**.**

The bird of paradise (*Strelitzia reginae* L.), native of South Africa, is one of the most commercialized flowers in Brazil and has an expressive commercial value. The species is a monocot and perennial flowering shrub and is commonly known as crane flower. It is an exotic specialty cut flower crop with flowers of brilliant colors and unusual appearance. The bird of paradise is cultivated on a commercial scale in many parts of the world like United States, South Africa, India, China and Brazil. Europe continues to be the prominent destination for floriculture exports (Sane et al., 2020).

However, the plant has a slow development, where mature plants propagated from seedlings start flowering only fourth year onwards (Kantharaju et al., 2008). Vegetative propagation can be performed dividing the clumps, resulting in a few number of seedlings. The propagation can also be performed with seeds, which presents dormancy and slow germination (Paiva et al., 2004). Each fruit is capable to produce an average of 30 seeds (Patel et al., 2017).

Seed propagation has an advantage because the species presents polymorphism, ensuring genetic variability for traits like plant height and flower size and length (Zheng et al., 2017). Thus, the use of seeds for the bird of paradise propagation allows exploration of genetic diversity in breeding programs. Genetic diversity of bird of paradise flower has been studied in Brazil (Santos et al., 2018), India (Basavarajappa et al., 2019 & Sane et al., 2020) and China (Zheng et al., 2017) to identify potential genotypes for breeding crosses.

The bird of paradise seed's tegument creates a barrier that difficult the embryo to exchange water with the environment, preventing the seed imbibition. For this type of dormancy, tegument scarification is recommended. There is also a possible physiological dormancy mechanism involved (Kumar et al., 2018) and the use of chemical substances like gibberellin can break this dormancy. Gibberellins, among other purposes, control various aspects of seed germination, including breaking dormancy and the mobilization of endosperm reserves (Marcos-Filho, 2015).

The biggest difficulty in *Strelitzia* propagation by seeds is the prolonged time and the low germination percentage. Thus, it is necessary to study methods that can enable the commercial production of seedlings from seeds, seeking efficient treatments to overcome dormancy, since there are several hypotheses to explain their origin (Patel et al., 2017). The aim of this research was to evaluate different methods to break the dormancy in bird of paradise seeds, using scarification and gibberellin.

Seeds of bird of paradise were collected in gardens and parks from the cities of Uberlândia and Uberaba from the Triângulo Mineiro region in Minas Gerais, with the climate of the region classified as Aw (tropical hot humid), according to the international classification of Köppen. It was collected mature fruits that started the opening process, moment considered as the seed physiological maturation point. After that, the aril of the seeds, located in the micropyle region, was totally removed to avoid fungi contamination.

The seeds were then homogenized and put in a solution of 0.1% sodium hypochlorite for 20 minutes for pathogen disinfestation. It was tested two methods of scarification with or without the addition of gibberellin (GA_3) totaling six dormancy break treatments: T_1- sandpaper in the micropyle region + 24 hours of imbibition with GA_3; T_2- sandpaper in the opposite region of the micropyle + 24 hours of imbibition with GA_3; T_3- no scarification + 24 hours of imbibition with GA_3; T_4 - no scarification + 24 hours of imbibition with water; T_5 - sandpaper in the micropyle region without imbibition; T_6 - sandpaper in the opposite region of the micropyle without imbibition. For the scarification, it was used a medium sandpaper (9 inch) where the seeds were scratched until reach the endosperm. The imbibition with gibberellin was performed with the addition of 500mg L^{-1} of GA_3.

The seeds were then displayed into gerbox boxes with two humified filter papers, where the seeds were sown between the two papers. It was used 50 seeds per plot (gerbox). The plots were placed in B.O.D germination chambers, in a temperature of 25±2 ^{o}C. Seed germination was accounted daily for 60 days, where the germination criteria was the radicle protrusion. During the experiment, when the filter paper was dry, it was moistened with distilled water.

The following germination variables were measured: time for first germination (t_0); time for the last germination (t_g); mean germination time ($\bar{t}$); germination percentage (*G*); dormancy percentage (*D*); dead seed percentage (*Ds*); mean germination rate ($\bar{v}$); germination rate (*GR*); uncertainty (*U*); and coefficient of variation of the

time (*CVt*) (Ranal & Santana, 2006).

The time for first germination was the number of days for the first germination, and the time for the last germination was the number of days for the last. Mean germination time ($\bar{t}$) was measured as: $\bar{t} = \sum_{i=1}^{k} n_i t_i / \sum_{i=1}^{k} n_i$; where t_i was the time from the start of the experiment to the i^{th} observation (day), n_i was the number of seeds germinated in time *i*, and *k* was the last time of germination. The coefficient of variation of the time was measured as $CV_t = \left(s_t / \bar{t}\right) 100$; where s_t was the standard deviation of the germination time. The mean germination rate was defined as: $\bar{v}(=1) / \bar{t}$ and the germination rate as: $GR = \sum_{i=1}^{k} n_i t_i$. The uncertainly was calculated using the relative frequency of germination (f_i) as: $U = \sum_{i=1}^{k} f_i log2 f_i$.

The data was submitted to Analysis of Variance after checking the assumptions of normality residuals and homoscedasticity by Shapiro-Wilk's and Levene's test, respectively, both tests with 0.01 of significance. If significant, means were compared by Tukey's test (*p-value*=0.05). The statistical analysis was performed in the R software version 3.5.2 (Core Team, 2019). After 60 days of experiment, the non-germinated seeds were submitted to tetrazolium test to verify their viability. For the tetrazolium test, it was used a solution of 0.1% of tetrazolium salt, based on the recommendation for *Glycine max* because of the lack of data for the studied species, to soak the seeds inside each gerbox during three hours in the B.O.D germination chamber with the 25±2 ^{o}C temperature. The temperature was chosen based on previous studies (Van De Venter, 1978 & Kumar et al., 2018). The seeds were then cut to evaluate the embryo viability.

Except for the time for the last germination and coefficient of variation of the time, all the variables presented significant differences by F test (*p-value*<0.05). The seeds with scarification in the micropyle region + 24 hours of imbibition with GA_3 (T_1) presented the best germinative characters (Table 1). In this treatment, the highest germination occurred (79.41%) compared to the other methods by Tukey's test, and only the treatment without scarification with gibberellin imbibition (64.71%) was considered significantly equal to that treatment. Seeds without a dormancy break treatment showed germination of 4.41%, confirming that the seeds have dormancy.

The efficiency of the treatment T_1 was confirmed in the other germinative traits. The seeds from this treatment started to germinate between the fourth and fifth day of the experiment. In the seeds without a dormancy break method (T_4), the germination started on the 17th day. In addition, the germination rate in T_1 was 5.85 seeds per day, statistically higher than the other treatments.

Although the treatment with only scarification in the micropyle region (T_5), presented an initial and average germination time significantly equal to T_1, the germination was lower (22.06%), showing that the dormancy involved in the species was not only physical, but a conjoint of physical and physiological dormancy. The mean germination rate is defined as the inverse of the mean germination time and showed that the scarification treatments in the micropyle region (T_1 and T_5) provided a faster germination of the seeds when the dormancy was broken (Table 1) but were significantly equal to the treatments with sandpaper scarification in the opposite region of the micropyle (T_2 and T_6).

It was observed that the germination of the species started with the radicle protrusion in the micropyle region. The primary root developed rapidly with the emission of several adventitious and secondary roots. The aerial part is then emitted from the same axis. The development of a normal seedling was established in seven days (Figure 1).

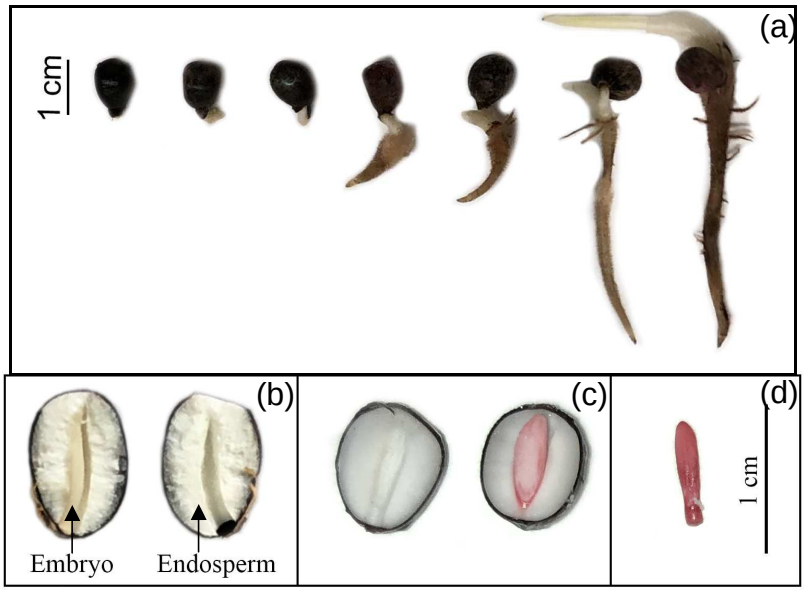
Table 1 - Germinative characters of *Strelitzia reginae* (L.) seeds submitted to different breaking dormancy treatments.

	t_0 (day)	t_g (day)	$\bar{t}$ (day)	*G* (%)	*Ds* (%)	*D* (%)
T_1	4.75 a	21.5	8.98 a	79.41 a	1.47 a	19.12 a
T_2	9.00 ab	*	9.00 a	1.47 d	10.29 a	88.23 b
T_3	21.75 c	30.0	26.21 b	64.71 ab	25.00 ab	10.29 a
T_4	17.25 bc	32.0	24.45 b	42.65 bc	50.00 b	7.35 a
T_5	5.50 a	28.0	10.10 a	22.06 cd	33.83 ab	44.12 ab
T_6	17.00 bc	*	17.00 ab	4.41 d	51.47 b	44.12 ab
CV(%)	25.72	18.82	20.08	15.93	22.41	27.64
F(Pr)	17.45 (<0.001)	3.02 (0.081)	18.54 (<0.001)	24.86 (<0.001)	2.895 (0.043)	8.82 (<0.001)

	$\bar{v}$ (day^{-1})	*CVt* (%)	*GR* ($seeds.day^{1}$)	*U*
T_1	0.341 a	57.87	5.85 a	2.06 a
T_2	0.326 ab	*	0.09 c	0.00 b
T_3	0.111 b	10.64	1.26 b	1.44 a
T_4	0.120 b	24.85	0.76 bc	1.64 a
T_5	0.341 a	41.93	1.26 b	1.01 ab
T_6	0.179 ab	0.00	0.18 bc	0.00 b
CV(%)	31.01	80.16	30.39	40.60
F(Pr)	8.62 (<0.001)	2.35 (0.113)	72.36 (<0.001)	10.00 (<0.001)

Means followed by the same letters, in the column, did not differ by Tukey's test with 0.05 of significance. t_0: time for first germination; t_g: time for the last germination; $\bar{t}$: mean germination time; *G:* germination percentage; *D*: dormancy percentage; *Ds*: dead seed percentage; $\bar{v}$: mean germination rate; *GR*: germination rate; *CVt*: coefficient of variation of the time; *U*: uncertainty. CV: Coefficient of variation. *Values not measured because of the low germination of the treatment. T_1- sandpaper in the micropyle region + 24 hours of imbibition with GA_3; T_2- sandpaper in the opposite region of the micropyle + 24 hours of imbibition with GA_3; T_3- no scarification + 24 hours of imbibition with GA_3; T_4 - no scarification + 24 hours of imbibition with water; T_5 - sandpaper in the micropyle region without imbibition; T_6 - sandpaper in the opposite region of the micropyle without imbibition.

Figure 1 - Seedling development of *Strelitzia reginae* (L.) along seven days (a); longitudinal section of the seed before (b) and after (c) tetrazolium test indicating its viability, with an embryo detail (d).



The scarification in the opposite region of the micropyle with gibberellin imbibition (T_2) presented low germinability because the cut favored the entry of pathogens that killed the seeds before they germinated (88.23% of the seeds were dead). Although the cut could favored the imbibition, it was performed on the opposite side which the radicle emerges, not helping in its emission, showing that the seed coat is quite rigid and blocks germination.

The treatment with soaking in water for 24 hours (T_4) and the scarification in the opposite region of the micropyle (T_6) had little effect in breaking the dormancy and were considered statistically equal. The treatments with scarification in the region of the micropyle (T_5) and the imbibition in GA_3 (T_3) showed intermediate behavior. However, the incidence of dead seeds in T_5 was higher, showing that scarification contributed to accelerate the dormancy break of some seeds but also favored the attack of pathogens by exposing the endosperm, which reinforces the need for a conjoint action of gibberellin and scarification in the dormancy break of the species. The treatment with the combined action of the two methods (T_1) obtained a dormancy percentage of 1.47% and 19.12% of dead seeds, statistically superior to the other treatments.

Although there are no records in the literature for the tetrazolium test to determine the viability of the seeds in Strelitziaceae species, the application of the methodology with a solution containing 0.1% of the tetrazolium salt for 3 hours in a temperature regime of 25 ± 2 ^{o}C was efficient in the coloring the living plant tissues (Figure 1) and can be applied in future researches.

Seed dormancy is a major problem to obtain seedlings from *Strelitzia* genus. Germination difficulty may be due the presence of inhibitors such as benzoic acid, cinnamic acid, coumarin, naringenin and abscisic acid (ABA). These compounds interrupt gene expression or promote the inhibition of enzyme complexes in the seed. Some growth regulators allow the blocking of these inhibitors such as indolacetic acid and gibberellins (Kumar et al., 2018). From the results found, it was observed that gibberellin was efficient in blocking these germination inhibitors.

The use of gibberellic acid has the purpose of breaking dormancy and accelerating seed germination, in addition to standardizing seed germination. Gibberellins, among other purposes, control various aspects of seed germination, including breaking dormancy and mobilizing endosperm reserves (Taiz & Zeiger, 2017). In our study it was possible to detect the positive effect of GA_3 in all analyzed germination characters, when associated with scarification.

There is few literature exploring dormancy break in *Strelitzia* species. Van De Venter (1978) verified a germination of 29% in bird-of-paradise seeds after eight weeks in 25 ºC, using as breaking dormancy method the immersion into sulfuric acid for two minutes. Singh (2006) also reported that treatment of *S. reginae* seeds with a solution of GA_3 at a concentration of 500 ppm for 48 hours alone and in conjunction with warm water at 50-55 °C for half hour improved seed germination. This research demonstrated the highest germination percentage for bird of paradise seeds (79.41%) using scarification with sandpaper in the micropyle region with 24 hours of imbibition with GA_3.

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