

Breaking dormancy in *Cycas revoluta*: A study of seed morphological characterization and dormancy mechanisms

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ABSTRACT: *Cycas revoluta* seeds exhibit both physical and morphological dormancy, posing significant challenges to their germination. This study investigated the morphological characteristics and dormancy mechanisms of *C. revoluta* seeds and evaluated methods to overcome these dormancies. Seeds were harvested over two consecutive years and subjected to different treatments. A morphological analysis of seed structure was conducted, along with assessments of physical (seed size, weight, and moisture content) and physiological tests (germination rate, germination speed, and mean time of germination). Morphological characterization revealed that the thick, woody sclerotesta forms an impermeable barrier, while the embryos within are often underdeveloped at the time of seed maturity. Germination of the first year demonstrated that removing the sclerotesta significantly enhances germination rates, with naked seeds achieving a 70% germination rate at 30 °C. In the second year, soaking naked seeds in gibberellic acid at 200 ppm for 24 hours further promoted germination, though its effect was modest compared to sclerotesta removal (naked seed). This study also demonstrated that storing seeds for a year allowed embryos to complete their development, further improving germination outcomes. These findings suggested that mechanical removal of the sclerotesta, combined with optimal storage conditions, is an effective strategy for overcoming dormancy in *C. revoluta* seeds and soak time in gibberellic acid (200 ppm) or water for 24 hours could enhance germination as well.

Index terms: embryo immaturity, morphological dormancy, ornamental, physical dormancy.

RESUMO: As sementes de *Cycas revoluta* apresentam dormência física e morfológica, o que representa desafios significativos para sua germinação. Este estudo investigou as características morfológicas e os mecanismos de dormência das sementes de *C. revoluta* e avaliou métodos para superar essas dormências. As sementes foram colhidas ao longo de dois anos consecutivos e submetidas a diferentes tratamentos. Foi realizada uma análise morfológica da estrutura das sementes, juntamente com testes físicos (tamanho, peso e grau de umidade das sementes) e fisiológicos (germinação, velocidade de germinação e tempo médio de germinação). A caracterização morfológica revelou que a espessa esclerotesta forma uma barreira impermeável, enquanto os embriões frequentemente estão subdesenvolvidos no momento da maturação das sementes. A germinação do primeiro ano demonstrou que a remoção da esclerotesta aumenta significativamente as taxas de germinação, com sementes descascadas alcançando 70% de germinação a 30 °C. No segundo ano, a imersão das sementes descascadas em ácido giberélico a 200 ppm por 24 horas promoveu ainda mais a germinação, embora seu efeito tenha sido modesto em comparação com a remoção da esclerotesta (semente descascada). Este estudo também demonstrou que o armazenamento das sementes por um ano permitiu que os embriões completassem seu desenvolvimento, aumentando ainda mais os resultados de germinação. Concluiu-se que a remoção da esclerotesta, combinada com condições ótimas de armazenamento, é uma estratégia eficaz para superar a dormência nas sementes de *C. revoluta*, e o tempo de imersão em ácido giberélico (200 ppm) ou em água por 24 horas também pode aumentar a taxa de germinação.

Termos de indexação: imaturidade do embrião, dormência morfológica, ornamental, dormência física.

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INTRODUCTION

Cycasds are among the most ancient and primitive seed plants still in existence today. They first appeared before the mid-Permian period and flourished with remarkable diversity during the Jurassic-Cretaceous era. Despite their ancient origins, the species we see today have evolved within the last 12 million years (Jones, 1993; Nagalingum et al., 2011; Zheng et al., 2017). Known as “living fossils” or evolutionary relics, Cycasds are invaluable to science due to their long evolutionary history and the insights they provide into the early development of seed plants (Chaw et al., 1997).

Cycasds serve as a food source in Japan, Australia, Southeast Asia, and the southern and eastern regions of India, providing starch extracted from the stems and seeds. Additionally, the wood is used to craft small boxes and dishes, while the leaves are woven into baskets, mats, and other items (Chahal, 2021; Li et al., 2023). *Cycas revoluta*, commonly known as the sago palm, king sago, sago Cycas, or Japanese sago palm, is native to China and Japan and is widely cultivated as a popular ornamental plant around the world (Forrester et al., 2020) including Brazil.

The flowering period occurs in summer, and the seeds reach full maturity in the fall (Du et al., 2024). This species can be propagated either by seed or by removing basal offsets, known as “pups.” The physical dormancy of the seeds causes a delay in germination, which may start within a few months and continue for up to a year or longer. The challenging germination process and slow growth rate increase the production costs of Cycas, leading to their scarcity (Zarchini et al., 2011; Frett, 1987).

Physical dormancy, caused by an impermeable seed coat (testa) or endocarp, prevents water from reaching the embryo and is a heritable trait. The testa and endocarp are derived from the ovule’s integuments and the ovary wall’s inner epidermal layer, respectively (Baskin and Baskin, 2014; Hudson et al., 2015). Several studies have explored methods to break the dormancy of *Cycas revoluta*. For instance, Frett (1987) tested varying soaking durations, pup removal, light and dark treatments, alternating temperatures (25/30 °C), and the application of gibberellic acid (500, 1000, or 5000 ppm). Benjelloun et al. (2021) tested warm stratification to promote embryo development and germination. Despite these efforts, germination in these studies was observed after approximately nine and five months, respectively.

Given the economic importance of *Cycas revoluta* in the ornamental plant market, developing effective methods to break physical dormancy and accelerate germination could significantly enhance plant production for commercial purposes and lower costs. Furthermore, a detailed description of the seed’s morphological characteristics could improve our understanding of its structure, potentially aiding in the refinement of dormancy-breaking techniques in the future. This study aims to evaluate the morphology of *Cycas revoluta* seeds and test various dormancy-breaking methods.

MATERIAL AND METHODS

The experiment was conducted at the Federal University of Lavras (UFLA) during 2017/2018. Seeds of *C. revoluta* were initially harvested in March 2017 from healthy female plants located around the campus, ensuring pollination by nearby male plants during summer. The seeds were stored (one year) in paper bags at room temperature (variation 25 to 30 °C) in the Seed Analysis Laboratory of the Seed Sector at UFLA. A second harvest took place in March 2018, using the same female plants for seed collection.

The study was divided into two experiments: one with seeds from the 2017 harvest (stored seeds) and the other with seeds from the 2018 harvest (fresh seeds).

Experiment One

This experiment tested three methods to break the physical dormancy of the stored *C. revoluta* seeds. The seed lot was homogenized and divided into three samples. The first sample was further divided and whole seeds soaked in water for 0, 48, 96, 144, and 192 hours at room temperature (~25 °C), with the water changed every 24 hours. The seeds were soaked in plastic bags, with enough water to cover all seeds. For the second sample, the seeds were cracked

using a hammer, the cracks were made vertically at the tip of the hilum. The purpose of cracking the sclerotesta was to allow water to enter the seed. In the third sample, both the sarcotesta and sclerotesta were removed using the same hammering technique leaving a naked seed. A Faxitron X-Ray machine with automated calibration of radiation intensity (Kvp) and exposure time (seconds) was also used to select only fully matured seeds, for the whole and cracked seeds, to ensure no empty or abnormal seed during the germination test.

Experiment Two

In this experiment, the sarcotesta and sclerotesta of the seeds were removed using the same technique as described previously. The seeds were homogenized, divided, and then soaked in gibberellic acid (GA) solutions at concentrations of 0, 50, 100, 200, 400, and 600 ppm for 24 and 48 hours. The seeds were soaked in plastic bags at room temperature (~25 °C), with the GA solution added until the seeds were fully covered. No solution changes were made during this experiment.

Seed quality assessment

Seed morphological characterization: High-quality images of whole seeds and naked seeds cut in half were captured using Groundeye® equipment with a high-resolution camera. Additionally, around 50 seeds were cut in half to observe embryo size changes. The maximum and minimum diameters of both fresh and stored seeds, with and without the sarcotesta/sclerotesta, were also measured using Groundeye® software, with results expressed in centimeters. X-rays of seeds in various sizes (~50 seeds) were taken to observe internal abnormalities using a Faxitron X-Ray machine with automated calibration of radiation intensity (Kvp) and exposure time (seconds). The Imaging analysis for morphological characterization involved a detailed examination of seed structure, with specific attention to identifying embryo presence, potential developmental abnormalities, and any deviations in seed morphology. All findings were qualitative, except for diameter measurements.

Seed Weight: Four replications of 50 whole seeds and naked seeds from 2017 and 2018 were weighed using a scale with 0.00 g precision, with results expressed in grams. The weight of individual seeds and one hundred seeds was determined.

Moisture content: Each replicate consisted of five whole seeds and naked seeds from both years (2017 and 2018), with four replications for each seed treatment. Seeds were cut into pieces smaller than 7.0 mm to facilitate moisture removal at this size, following the guidelines of the Brazilian Rules for Seed Testing (RAS). The seeds were then dried in an oven at 105 °C ± 3 °C for 24 hours (Brasil, 2009). The results were expressed as a percentage.

Germination: Four replications of 25 seeds were sown at a depth of half the seed (horizontally) in plastic trays (45 cm length x 20 cm width x 10 cm depth) containing a 2:1 mixture of soil and sand. Trays from experiment one was placed in growth chambers at 25 °C and 30 °C. In the experiment two, germination trays were placed in a growth chamber at 30 °C. Germination was counted daily for up to 90 days (Figure 1A). Results were expressed as percentages of normal seedling after 90 days (shoot and root).

Speed of seed germination: it was assessed alongside the germination test by counting the number of emerged hypocotyl (Figure 1B) daily. The germination speed index was calculated using Maguire (1962) formula.

Mean time of germination: performed with the germination test and calculated using the Zhang et al. (2014) species may germinate at different times so as to mitigate competition and to take advantage of different aspects of the seasonal environment (temporal niche differentiation formula. It was counted seedlings with presence of shoot and root (Figure 1B).

Statistical analysis were performed using SISVAR software (Ferreira, 2014). The analyses included analysis of variance (ANOVA) and comparison of means at a 5% significance level using the Scott-Knott test (for germination, speed of germination, and mean time of germination) and Tukey's test (for moisture content, maximum and minimum diameters). Data from the physical analysis (moisture content, maximum diameter, minimum diameter, seed weight,



Figure 1. *Cycas revoluta* (naked seeds) seedlings at approximately 15 days (A) and 90 days (B) after sowing.

and weight of one hundred seeds) were compared between the two years to observe physical changes in the seeds, except for naked seed weight, which was averaged across both years as the weight did not differ significantly.

RESULTS AND DISCUSSION

Morphological Characterization

Cycas revoluta seeds were found to be large, ovoid to oblong in shape, often slightly flattened on both sides (Figure 2). The seed coat, known as the sclerotesta, was thick, woody, and tough, offering significant protection to the developing embryo by creating a physical barrier to water absorption. Surrounding the hard testa was a fleshy layer called the sarcotesta, which was soft, somewhat oily, and displayed a rich orange to red color. The hilum, a small circular scar located near one end of the seed, was the point where the seed was attached to the ovary during development. It was usually a lighter color than the surrounding seed coat, often pale brown or tan. Inside the seed coat, *C. revoluta* seeds contained a large starchy endosperm that served as a food reserve for the developing embryo. The endosperm was white to creamy in color and had a firm texture.

An intriguing observation during the morphological characterization was the presence of a suspensor structure attached to the embryo (Figure 2). This structure is responsible for pushing the developing embryo deeper into the endosperm, providing nutrition and growth regulators (Schwartz et al., 1997).

Moreover, the presence or absence of embryos within the endosperm tissue was noted (Figure 3), a phenomenon also reported by Lima et al. (2018) in the germination of açai seeds. Figueiredo and Köhler (2018) explain that imbalances

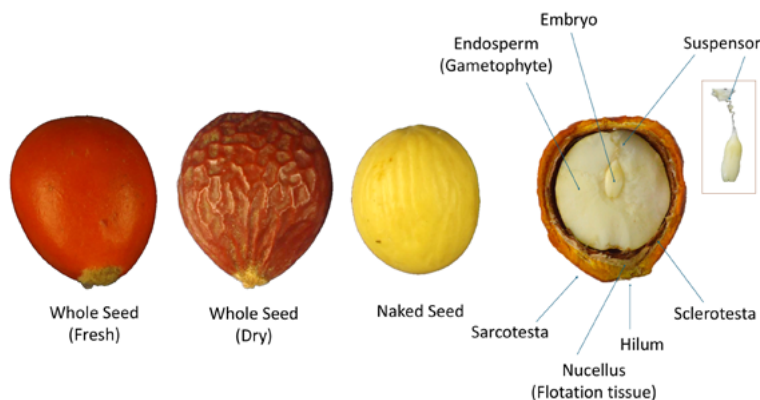


Figure 2. Morphology of *C. revoluta* seed.

in auxin levels can lead to abnormal seed development, including the formation of seeds without embryos. Variations in embryo sizes were also observed, supporting Benjelloun et al. (2023) affirmation that *C. revoluta* seeds are immature post-ripening and require additional time to fully develop. This explains the differing stages of embryo development observed in this study.

The results for moisture content showed higher moisture levels in freshly harvested whole seeds from 2018 compared to the dried whole seeds from 2017 (Figure 2, Table 1). However, once the sarcotesta and sclerotesta were removed, the naked seeds did not show significant differences in moisture, indicating the effective impermeability of the sclerotesta in creating physical dormancy. Physical dormancy occurs when a hard, impermeable seed coat or endocarp prevents water from reaching the embryo, inhibiting germination. This trait, often heritable and controlled by the testa or endocarp, likely evolved to help plants survive in unpredictable and harsh environments, potentially increasing their resilience to future climate variability (Hudson et al., 2015). The maximum and minimum diameters of whole seeds were approximately 4.00 cm and 3.00 cm, respectively (Table 2), with seeds from 2017 being slightly larger, likely due to more favorable conditions. However, comparisons of individual seed weight and the weight of 100 seeds showed that 2017 seeds had a significantly lower weight, indicating moisture loss during storage (Figure 2). In contrast, naked seeds from both years showed no differences in diameter or weight, as the sarcotesta and sclerotesta provide an impermeable barrier around the embryo, preventing moisture loss over time and maintaining consistent embryo size between years. The results shown for naked seeds are presented as an average of both years, as no significant differences were observed when analyzed separately.



Figure 3. Presence and absence of embryos in *C. revoluta* seeds. Presence of embryo (A), presence of suspensor and absence of embryo (B), and absence of embryo (C).

Table 1. Moisture content (MC) of the whole seed (WS, %) and naked seed (NS, %) of *C. revoluta* obtained from stored and fresh harvested seeds

Seed	MC	
	2017	2018
WS	34.97 bB*	52.66 aA
NS	47.44 aA	48.08 aA
C.V. (%)	3.87	
Mean (%)	45.78	

*Means followed by same lowercase letters in the column and uppercase letters in the row do not differ by Tukey test at 5% of probability. C.V. - coefficient of variance.

Table 2. Results of maximum diameter (DMAX, cm), minimum diameter (DMIN, cm, seed weight (SW, g) and weight of one hundred seeds (W100, g) obtained from Whole seed (2017 and 2018) and naked seed of *Cyca revoluta*.

Treatment	DMAX	DMIN	SW	W100
Whole seed (2018)	4.03 b*	3.26 b	18.88 a	1846.6 a
Whole seed (2017)	4.13 a	3.47 a	14.45 b	1474.2 b
Seed	2.987 c	2.56 c	10.01 c	1003.3 c
C.V. (%)	1.15	0.84	0.96	1.93
Mean (%)	3.72	3.09	14.45	1441.3

*Means followed by same letter in the column do not differ from Tukey test at 5% of probability. C.V. - coefficient of variance.

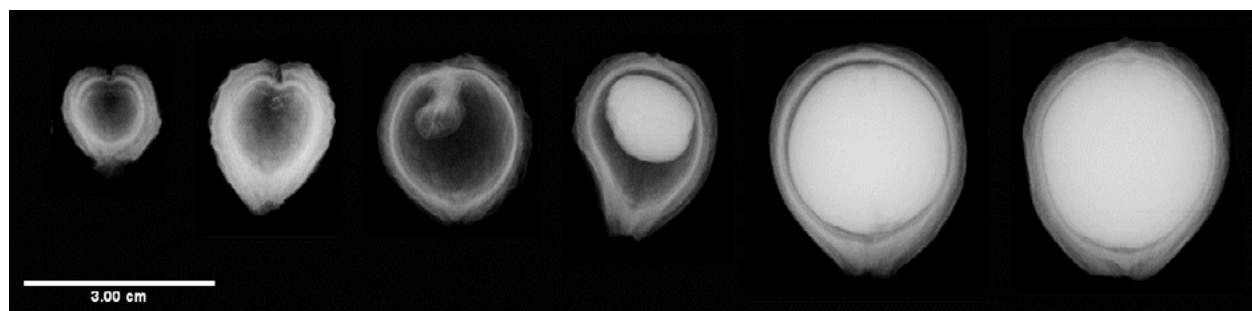


Figure 4. X-rays of *C. revoluta* showing empty seeds, abnormal seeds, and fully developed seeds. 2017.

The X-ray evaluation (Figure 4) provided insights into seed development within the sclerotesta. It was evident that smaller seeds were often empty or exhibited abnormal development. X-ray technology is highly useful as a non-destructive approach to select fully developed seeds, potentially improving seed lot quality by removing seeds with abnormalities (Medeiros et al., 2020; Musaev et al., 2022).

Breaking physical dormancy (Experiment one)

Germination results from the stored seeds harvested in 2017 (Figure 5A) demonstrated that temperature significantly influenced germination across all treatments, with 30 °C being the optimal temperature for *C. revoluta* seed germination. Temperature is a key factor triggering germination, and each species has its optimal temperature based on its region of origin (Dürr et al., 2015). *C. revoluta* is primarily distributed across the Ryukyu Islands (Japan), a subtropical region where maximum temperatures can reach 30 °C (Chang et al., 2023).

Soaking time also impacted germination. At 25 °C, soaking seeds for 48 hours increased germination compared to the control (0 hours), while extended soaking times led to decreased germination. However, at 30 °C, all soaking times improved germination, with 192 hours showing a twofold increase compared to the control. Cracking the sclerotesta increased germination by 2.6 times, and removing the sclerotesta entirely increased germination by 3.5 times at 30 °C. At 25 °C, the results were similar to the soaking treatment, but naked seeds exhibited the highest germination rates at both temperatures (44% at 25 °C; 70% at 30 °C), highlighting the importance of sclerotesta removal in breaking physical dormancy in *C. revoluta* seeds.

Previous studies have explored methods to overcome dormancy in *C. revoluta* to enhance germination. For instance, Frett (1987) obtained germination of 47% using sulfuric acid scarification, while Zarchini et al. (2011) achieved 60% germination with sulfuric acid treatment on seeds without sarcotesta. These same authors also tested hot water, achieving 63% germination with water at 100 °C. However, the control showed 83% germination, what shows that the treatments (sulfuric acid and hot water) damaged the seeds. More recent research by Benjelloun et al. (2021; 2023)

applied warm storage treatment (30 °C for two months), resulting in a maximum germination rate of 50%. Although in some of these researched methods germination was improved, the process still took 100 to 200 days, a significant period for germination. In the ornamental market, the long germination period of *C. revoluta* seeds delays production and plant availability, reducing the market's ability to meet consumer demand quickly. This slow turnaround can lead to higher costs for growers, lower profitability, and potential loss of market share to faster-germinating competitors.

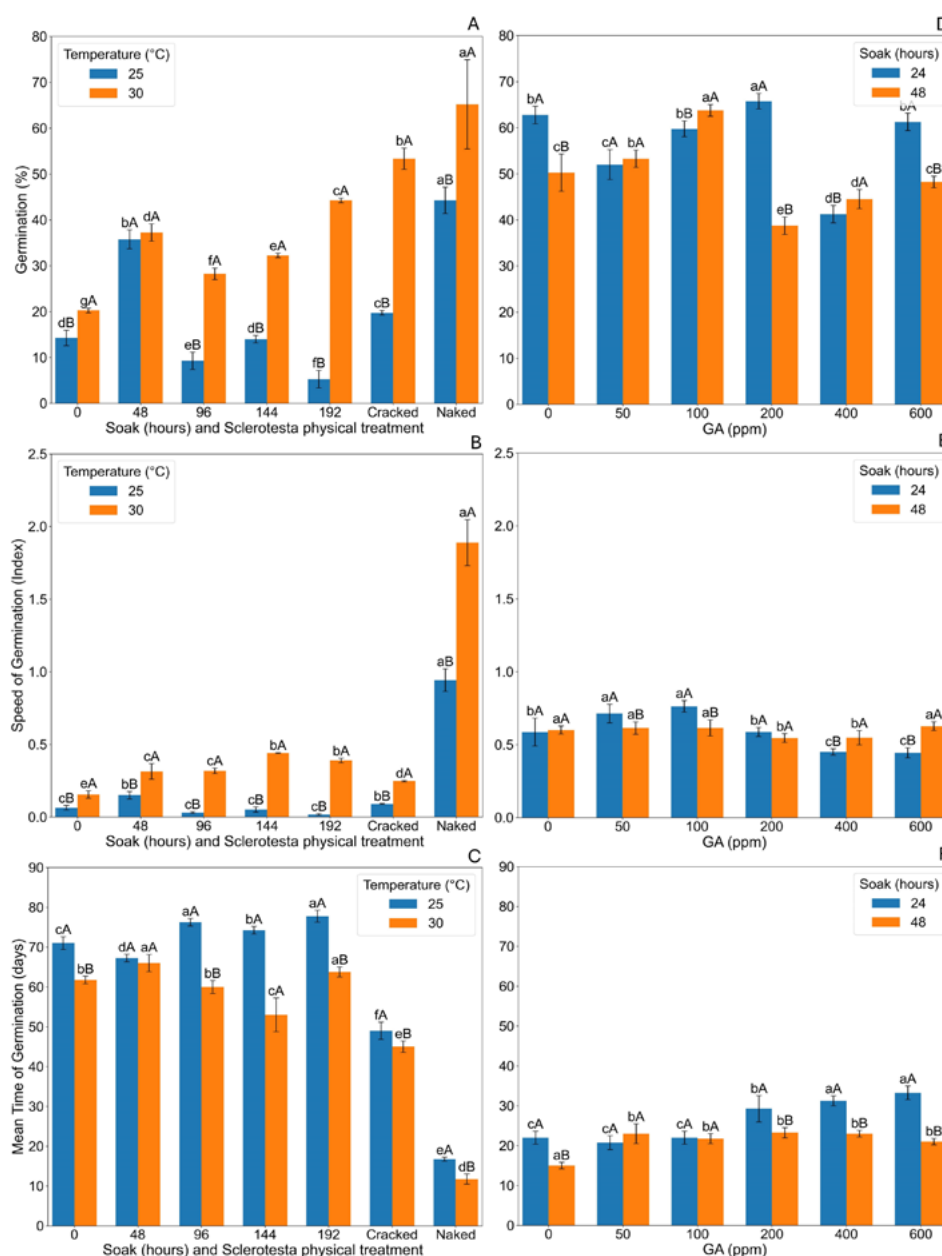


Figure 5. Germination (A-D,%), Speed of Germination (B-E, index), Mean Time of Germination (C-F, days) results of stored seeds (2017) of *C. revoluta* seeds soaked in water for 0, 48, 96, 144 and 192 hours, and cracked and naked seeds, incubated in two different temperatures (25 °C and 30 °C) (A, B, and C) and fresh seeds (2018) soaked in 0, 50, 100, 200, 400 and 600 ppm of gibberellic acid for 24 and 48 hours (D, E, and F). Means followed by same lower-case letter (compared in each temperature separately) and capital letter (compared between temperatures) do not differ from the Scott-Knott test at 5% of probability. Coefficient of variance: A-5.72%, B-14.10%, C-3.13%, D-4.12%, E-8.08%, and F-7.16%.

The speed of germination is crucial as it enables seedlings to establish quickly, taking advantage of favorable conditions before they change. Our results (Figure 5B) showed that naked seeds at 30 °C had the highest germination speed compared to all other treatments, with a speed 30 times greater than that of the control seeds. Faster germination reduces the risk of predation and disease, and helps plants outcompete neighboring species for resources like nutrients and space (Reed et al., 2022).

The mean time of germination (Figure 5C) further underscored the importance of removing the sclerotesta, as *C. revoluta* seeds germinated in 12 days at 30 °C and 17 days at 25 °C - five times faster than the control treatment (Figure 1). No existing research was found that successfully broke *C. revoluta* dormancy in such a short time frame.

C. revoluta seeds exhibit morphological dormancy, characterized by a slow embryo development, followed by mechanical dormancy that creates impermeability to water uptake. Dehgan and Schutzman (1989) asserted that *C. revoluta* seeds will eventually germinate under optimal conditions. However, gibberellin acid could act as a promoter for triggering germination (Shu et al., 2016) and fasten germination.

Influence of Gibberellic Acid (Experiment two)

The second experiment aimed to determine the influence of GA on *C. revoluta* seed germination. The results (Figure 5D) showed that soaking seeds in a 200 ppm GA solution for 24 hours resulted in the highest germination rate of 66%, though this was only a 5% improvement over the 24-hour control treatment. This suggests that soaking in water alone for 24 hours is sufficient to promote germination in freshly harvested *C. revoluta* seeds. The 24-hour soaking time was effective across all treatments, except for 100 and 400 ppm GA. Germination speed (Figure 5E) was significantly higher in the 50 ppm and 100 ppm GA treatments, though no significant differences were observed across treatments, with speeds ranging from 0.5 to 0.7 on the index scale. This slower speed of germination compared to Experiment One (naked seeds) (Figure 5B) may be related to the embryo development during imbibition. This highlights the importance of storing *C. revoluta* seeds before planting to allow for embryo maturation, leading to more uniform germination and higher speeds. Dresch et al. (2014) confirmed the positive effect of storage on species with immature embryos post-ripening and the use of GA to promote germination. Combining these two methods could help achieve higher seed germination rates in *C. revoluta* than observed in this research.

The mean time of germination (Figure 5E) in the second experiment showed that, within 24 hours, the fastest germination occurred in 22 days with 0, 50, and 100 ppm GA, and in 15 days in the 48-hour control treatment. These results indicate that immature embryos may delay germination by a few days compared to stored seeds from Experiment One, where the best treatment (naked seeds) had a mean germination time of approximately 10 days.

The two experiments demonstrated that removing the sclerotesta of the seed significantly breaks physical dormancy, while storage and the use of gibberellic acid (GA) serve as secondary tools to assist in embryo development and promote germination. The most effective way to break the sclerotesta without damaging the seeds is by applying vertical pressure (hammering) at the hilum part of the seed. This research could support the development of machinery designed to remove the sclerotesta from *C. revoluta* seeds, thereby reducing the germination time, which is a major bottleneck for producers.

CONCLUSION

Cycas revoluta seeds exhibit both physical and morphological dormancy. The sclerotesta, a hard seed coat, creates an impermeable barrier that hinders water absorption.

Dormancy in *Cycas revoluta* can be effectively overcome by removing the sclerotesta, which, along with a temperature of 30 °C, significantly improves germination. Additionally, storage provides the necessary time for the embryo to complete its development. However, further studies are recommended to determine the minimum storage duration required before optimal germination can occur.

The soaking time in gibberellic acid 200 ppm can enhance germination in naked *Cycas revoluta* seeds when applied for 24 hours.

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