Greenhouse Plant Production Journal

Effects of Chemical and Hormonal Priming on Seed Dormancy Release, Germination, and Early Seedling Growth of *Cycas revoluta*

Amir Gholamian[a], Sadegh Mousavi-Fard[a]*, Saeed Reezi[a]

[a]Department of Horticultural Science, Faculty of Agriculture, Shahrekord University, Shahrekord, Iran

Original Article

Use your device to scan and read the article online

Citation: Gholamian, A., Mousavi-Fard, S. and Reezi, S. 2027. Effects of Chemical and Hormonal Priming on Seed Dormancy Release, Germination, and Early Seedling Growth of *Cycas revoluta*. Greenhouse Plant Production Journal 4(1): 1-11.

https://doi.org/10.22034/jgpp.2026.2110222.1127

KEYWORDS

Dormancy-breaking
Gibberellic acid (GA_3)
Potassium nitrate (KNO_3)
Sulfuric acid (H_2SO_4)
Sclerotesta

ARTICLE HISTORY

Received: 27October 2026
Revised: 14 November 2026
Accepted: 30 December 2026

ABSTRACT

Cycas revoluta seeds have several dormancy barriers, including a fleshy sarcotesta, hard sclerotesta, and partial embryo immaturity, that delay germination for up to nine months. This study evaluated combined chemical and hormonal priming as a non-thermal strategy to overcome these constraints, assessing germination under different substrates. The most effective treatment among those tested, sulfuric acid (15 M) for 30 min, gibberellic acid (GA_3; 500 ppm) for 24 h, and potassium nitrate (KNO_3; 3%) for 24 h, significantly enhanced germination ($P < 0.05$), achieving 86.67% in perlite–cocopeat. No germination occurred in any treatment in the soil-based substrate consisting of equal volumes of sand, soil, and well-decomposed farmyard manure. The contrasting germination outcomes indicate that successful germination under the conditions of this experiment was observed only in the perlite–cocopeat substrate; however, the mechanisms underlying this difference remain undetermined. The combined treatment likely involved sulfuric acid-mediated seed coat weakening, GA_3-associated stimulation of embryo metabolism, and KNO_3-related metabolic activation, although these mechanisms were not directly investigated. No visible embryo damage was observed based on visual inspection only. The same treatment produced the highest values for several growth-related traits, including root volume (26.47 cm^3), leaf area (63.33 cm^2), and plant height (23.40 cm). However, chlorophyll content index (CCI) did not differ significantly among treatments. Overall, H_2SO_4 (15 M, 30 min) + GA_3 (500 ppm, 24 h) + KNO_3 (3%, 24 h), when combined with a suitable soilless substrate, improved germination and several early seedling growth traits under the conditions of this experiment, although no significant improvement in CCI was detected.

* Corresponding author: S. Mousavi-Fard
E-mail address: sadeghmosavifard@gmail.com ; mousavifard.s@sku.ac.ir

Publisher: Arak University

1. Introduction

Cycas are widely valued ornamental plants cultivated in tropical and subtropical regions because of their distinctive morphology, slow growth habit, and striking architectural form (Marler, 2025). Among them, *Cycas revoluta* Thunb. (Cycadaceae) is one of the most widely cultivated cycad species worldwide, particularly in East and South Asia, including Japan, China, and India. Its tolerance to drought and moderate low temperatures, together with its stiff, leathery foliage, has made it highly desirable for both indoor decoration and outdoor landscape plantings (Marler, 2026).

Seed propagation remains the principal method for large-scale production of *Cycas revoluta*, as it enables efficient multiplication and the production of plants suitable for horticultural use. However, germination of *C. revoluta* seeds is often slow and non-uniform due to seed-related characteristics that may impose physical and developmental constraints on germination. One major barrier is the sarcotesta, an orange fleshy outer layer that contains waxes and lipids and has been associated with the presence of germination-inhibiting substances. Retention of the sarcotesta has been reported to reduce germination percentage and uniformity while increasing the risk of seed deterioration during storage and sowing (Dehgan and Schutzman, 1989; Marler, 2026). Another constraint is the sclerotesta, a thick and mechanically resistant seed coat that protects the embryo from environmental stress but may also restrict water entry and embryo emergence. In addition, *C. revoluta* seeds may exhibit delayed germination associated with incomplete embryo development at the time of seed dispersal, requiring a post-harvest period for further embryo development before germination can occur (Dehgan and Schutzman, 1989; Calonje *et al*., 2011).

Several approaches have been investigated to overcome seed-related constraints in *Cycas* seeds and improve propagation efficiency. Conventional methods typically begin with removal of the sarcotesta by soaking seeds in water at 25 ± 2 °C for 48–72 h, which softens the fleshy layer and facilitates its removal while reducing the risk of decay. Surface sowing has also been reported to enhance aeration and gaseous exchange around the sclerotesta, thereby creating more favorable conditions for the initiation of metabolic processes associated with germination and reducing the likelihood of fungal infection (Benjelloun *et al*., 2021; Corbineau, 2022).

To overcome the potential restriction imposed by the sclerotesta, chemical scarification using concentrated sulfuric acid has been widely employed. Sulfuric acid facilitates partial degradation of structural components of the seed coat, thereby potentially increasing its permeability to water and oxygen (Paparella *et al*., 2015). Previous studies have shown that such treatments can substantially improve germination. For example, Zarchini *et al*. (2011) reported germination rates of up to 95% following sulfuric acid treatment combined with boiling-water exposure. However, the use of elevated temperatures and highly concentrated acid may increase the risk of embryo injury and reduce seed viability.

In addition to physical scarification, plant growth regulators play an important role in regulating seed dormancy and germination. Gibberellins, particularly gibberellic acid (GA_3), are involved in the regulation of seed germination and may counteract the inhibitory effects of abscisic acid (ABA), stimulate hydrolytic enzyme activity, and promote embryo growth through enhanced metabolic activity and cellular expansion (Nonogaki, 2019; Saeidi *et al*., 2020). Historically, early investigations demonstrated that *Cycas revoluta* seeds exhibit delayed embryo development and dormancy-related characteristics, wherein embryos may require a prolonged post-harvest period before completing their development (Dehgan and Schutzman, 1989). Subsequent studies explored hormonal supplementation to accelerate this process; exogenous gibberellic acid (GA_3), in particular, has been associated with the regulation of ABA-related dormancy responses and the mobilization of stored reserves in germinating seeds (Nonogaki, 2019). In gymnosperms such as *C. revoluta*, these reserves are stored in the female gametophyte rather than in a true endosperm. For instance, Lima and Oliveira (2024) demonstrated that soaking *C. revoluta* seeds in GA_3 solutions (200 mg L^{-1}) could improve germination responses compared with untreated seeds. Similarly, Zarchini *et al*. (2011) examined combined physical and chemical treatments and showed that sulfuric acid scarification followed by thermal treatment significantly elevated final germination to above 80%, albeit with prolonged exposure times that may increase the risk of embryo injury. Alongside gibberellins, nitrogenous compounds, particularly potassium nitrate (KNO_3), have emerged as potent germination stimulators via nitrate-sensing and phytochrome-mediated signaling networks (Duermeyer *et al*., 2018). KNO_3 treatments have also been investigated as a means of improving germination in different plant species, although their effectiveness and underlying physiological responses may vary among species and experimental conditions (Bakhshy *et al*., 2026).

Despite these advances, further investigation is needed to determine whether combining chemical scarification with hormonal and nitrate treatments can improve germination and early seedling establishment in *Cycas*. Therefore, the primary objective of this study was to evaluate the efficacy of chemical (H_2SO_4, KNO_3) and hormonal (GA_3) priming combinations in overcoming seed dormancy and inducing germination of *C. revoluta* across two contrasting substrates (a native dryland soil vs. an aerated soilless mix), and subsequently to characterize early seedling growth and biomass allocation under successful establishment conditions. We

hypothesized that the combined application of chemical and hormonal treatments would improve germination responses, while growing substrate conditions would influence germination and subsequent seedling establishment.

2. Materials and Methods

2.1. Experimental Design and Plant Material

The experiment was conducted during 2023–2024 in the research greenhouses of Shahrekord University, Iran, to investigate the effects of combined chemical scarification and hormonal priming on the germination performance of *Cycas revoluta* seeds. Mature seeds were collected in December 2023 from healthy mother plants grown in commercial ornamental plant nurseries in Tonekabon, northern Iran. Following harvest, the seeds were packed in breathable cotton bags, shipped by post to the laboratory, and stored under dry, well-ventilated conditions (18 ± 2 °C and 45–50% relative humidity) until the experimental preparation commenced.

Prior to treatment (approximately one month before sowing), seeds underwent a preparation phase and initial screening. First, a preliminary screening was performed using a water flotation test. Buoyant, hollow, or aborted seeds were discarded, and only fully sunken seeds were retained. This flotation test was used only as a preliminary screening method and represents a crude proxy for seed condition; it does not confirm embryo viability. The selected seeds were placed in mesh bags and soaked in tap water for two weeks to soften the sarcotesta. To maintain adequate oxygen availability and prevent anaerobic conditions, the soaking water was replaced every 8 h. After softening, the orange fleshy sarcotesta was manually removed, and the exposed seeds were visually inspected to eliminate any mechanically damaged specimens. Following the application of the dormancy-breaking treatments, the seeds were sown into the experimental substrates in early spring. Before sowing, seeds, pots, substrates, and greenhouse equipment were disinfected with a broad-spectrum surface sanitizer (sodium hypochlorite) to minimize fungal and bacterial contamination.

Initially, seven treatments were evaluated, including an untreated absolute control (sarcotesta-free seeds receiving no chemical or hormonal treatment), an acid-priming control (H_2SO_4 alone), and five combined priming treatments: (1) H_2SO_4 + GA_3 (500 ppm) + KNO_3 (3%), (2) H_2SO_4 + GA_3 (200 ppm) + KNO_3 (3%), (3) H_2SO_4 + GA_3 (500 ppm), (4) H_2SO_4 + GA_3 (200 ppm), and (5) H_2SO_4 + KNO_3 (3%). The experiment was laid out in a completely randomized design (CRD) with three replications, each comprising five pots, with one seed per pot (15 seeds per treatment). Germination percentage was calculated separately for each of the three original replications based on the number of germinated seeds relative to the five seeds initially sown in that replication. Following the germination assessment, one pot containing a successfully germinated seedling was selected from each of the three original replications per treatment for subsequent seedling growth measurements. Thus, three individual pots, corresponding to the three original replications, provided the observations used for the statistical analysis of seedling growth traits. Because seeds in both the untreated absolute control and the H_2SO_4-priming-only treatment completely failed to germinate (0% germination capacity) owing to the complex morphophysiological and physical dormancy characteristic of *C. revoluta*, they produced zero variance and were consequently omitted from subsequent quantitative and statistical analyses of seedling growth traits.

Seed treatments were applied sequentially. Chemical scarification was first performed by immersing the seeds in a sulfuric acid solution (15 M, prepared by dilution of analytical-grade 98% H_2SO_4) for 30 min with gentle stirring to avoid excessive heat accumulation and potential embryo injury. After scarification, the seeds were thoroughly rinsed with distilled water. Hormonal priming was then conducted by soaking seeds in GA_3 solution, either 500 ppm for 24 h or 200 ppm for 48 h. For treatments including potassium nitrate, seeds were subsequently immersed in a 3% KNO_3 solution for 24 h. During all soaking stages, the solutions were replaced every 8 h to maintain oxygen availability and prevent anaerobic conditions.

Following treatment, seeds were sown in plastic pots (2 L; 14 cm diameter) filled with either a soil-based substrate consisting of an equal-volume mixture of sand, soil, and well-decomposed farmyard manure (1:1:1, v/v/v), or a soilless substrate consisting of perlite and cocopeat (1:2, v/v). All treatments, including the untreated and H_2SO_4-only controls, were subjected to the same sowing and growing conditions. The physicochemical properties of the soil used in the soil-based substrate are presented in Table 1. Seeds were sown at a depth of 4 cm in the respective substrates. Pots were maintained in a controlled greenhouse with daytime temperatures of 28–32 °C (reaching up to 35 °C during peak solar hours), nighttime temperatures of 25–27 °C, and relative humidity of 70–80%. This thermal regime was selected based on previous findings showing that 30 °C is optimal for *Cycas revoluta* seed germination, promoting significantly higher germination rates and speeds compared to 25 °C (Dehgan and Schutzman, 1983; Lima and Oliveira, 2024). Furthermore, maintaining nighttime temperatures close to diurnal levels (~25–27 °C) ensured continuous warm stratification, supporting ongoing embryo development and reflecting the species' native subtropical climate.

Table 1. Physicochemical properties of the soil used for pot cultivation.

Soil property	Value	Unit
pH	7.18	–
Electrical conductivity (EC)	0.57	dS m^{-1}
Organic carbon (OC)	1.07	%
Total neutralizing value (TNV; lime)	17.5	%
Field capacity (FC)	28	%
Nitrogen	0.12	%
Phosphorus	58.5	mg kg^{-1}
Potassium	348.8	mg kg^{-1}
Sodium	9.5	mg kg^{-1}
Calcium	5000	mg kg^{-1}
Texture	Clay loam	–

2.2. Germination percentage

Seed germination was monitored throughout the experimental period. Germination percentage (GP) was calculated separately for each replicate as the proportion of germinated seeds relative to the total number of seeds sown in that replicate, using the following equation:

$GP_i = (G_i / N_i) \times 100$

where G_i represents the total number of seeds germinated in replicate i, and N_i represents the total number of seeds sown in the same replicate. The mean germination percentage was then calculated from the three replicate values, and the replicate mean was considered the experimental observation for statistical analysis. (Gonçalo *et al*., 2026). Germination timing was recorded as the time elapsed from sowing until germination was first observed in each successful treatment.

2.3. Seedling growth traits

Seedling growth parameters were measured at the end of the three-month experimental period. The seedlings from the three selected pots per treatment were carefully removed from the substrate, and their roots were gently washed with water to remove adhering soil, perlite, and cocopeat particles. The harvested seedlings were then used for destructive growth measurements. Seedlings were separated into shoot and root components. Fresh biomass of shoots and roots was measured using a precision analytical balance. Root length was measured from the hypocotyl–root junction to the tip of the primary root, while plant height was determined from the substrate surface to the apical meristem using a graduated ruler. Hypocotyl diameter, an indicator of early seedling vigor, was measured at its widest point using a digital caliper (Lima and Oliveira, 2024).

Leaf morphological traits included the number of fully expanded true leaves, petiole length of the first and second leaves (measured separately from the point of attachment to the stem to the base of the leaf blade), leaf length (measured from the point of petiole attachment to the lamina tip), and leaf width (measured at the widest part of the lamina). Leaf area was determined using Digimizer image analysis software and expressed in square centimeters (cm^2). Root volume was estimated using the water displacement method by immersing the root system in a graduated cylinder and recording the volume of displaced water.

Physiological status was assessed by estimating the Chlorophyll Content Index (CCI) using a portable Chlorophyll Content Meter (CCM-200, Opti-Sciences Inc., Hudson, NH, USA), which provides a non-destructive Chlorophyll Content Index (CCI) based on optical transmission ratios at red (653 nm) and near-infrared (931 nm) wavelengths. For dry biomass determination, shoot and root samples were oven-dried for 48 h and weighed using a precision balance. Individual seed weight was also recorded prior to treatment application using an analytical balance (Gholamzadeh Alam *et al*., 2022.)

2.4. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) within a completely randomized design (CRD) to evaluate the effects of chemical scarification and hormonal priming treatments on germination and seedling traits. Prior to ANOVA, the statistical assumptions of normality and homogeneity of variances were verified using the Shapiro–Wilk test (along with normal Q–Q plots) and Levene's test, respectively. Mean comparisons were performed using the Fisher's Protected least significant difference (PLSD) test at a significance level of $p \leq 0.05$. Results are presented as mean $\pm$standard error (SE) based on three replications. Statistical

analyses were conducted using Minitab software (Version 21, Minitab Inc., USA), and figures were generated using Microsoft Excel. Significant differences among treatments are denoted by different letters in the tables and figures.

3. Results

3.1. Seed germination

Variance Analysis (ANOVA) revealed that priming treatments significantly influenced the germination of *Cycas revoluta* ($P < 0.05$), indicating that the applied treatments affected germination performance and dormancy alleviation. However, no germination was observed in any of the priming treatments when seeds were sown in the soil-based substrate consisting of an equal-volume mixture of sand, soil, and well-decomposed farmyard manure (1:1:1, v/v/v). Importantly, the absence of germination in this substrate did not appear to be related to the inability of the seeds to germinate, as germination occurred successfully in the second, soilless substrate consisting of perlite and cocopeat (1:2, v/v). Although differences in aeration, water retention, and drainage between the two substrates may have contributed to this response, their physical properties were not measured, and the mechanisms underlying the observed difference could not be determined. Therefore, the following results refer to seeds grown in the perlite–cocopeat substrate.

Marked variation in germination percentage was observed among the evaluated priming treatments. Germination of treated seeds commenced approximately 85 days after sowing and continued over the subsequent week (85–92 days after sowing). Among the treatments, H_2SO_4 + GA_3 (500 ppm) + KNO_3 produced the highest germination percentage (86.67%) (Fig. 1), whereas H_2SO_4 + KNO_3 resulted in the lowest germination percentage (20%).

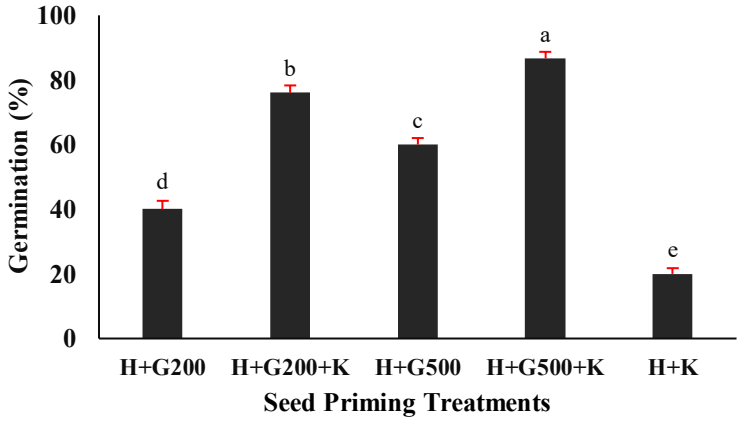


Figure 1. Effect of seed priming treatments on the germination percentage of *Cycas revoluta*. Treatments included H+G200 (H_2SO_4 + GA_3, 200 ppm), H+G200+K (H_2SO_4 + GA_3, 200 ppm + KNO_3), H+G500 (H_2SO_4 + GA_3, 500 ppm), H+G500+K (H_2SO_4 + GA_3, 500 ppm + KNO_3), and H+K (H_2SO_4 + KNO_3). Values are presented as mean ± SE. Different letters above the bars indicate significant differences among treatments according to the LSD test at $P < 0.05$.

3.2. Morphological traits

Priming treatments had a significant effect on root and shoot traits ($P < 0.05$; Table 2). The treatment of H_2SO_4 + GA_3 500 ppm + KNO_3 produced the highest values for leaf area (63.33 cm^2), leaf length (8.07 cm), plant height (23.40 cm), and hypocotyl diameter (1.60 mm) (Figs. 2 and 3). The first petiole length ranged from 7.67 to 11.00 cm across the treatments, with the highest value recorded under H_2SO_4 + GA_3 (500 ppm) treatment. Second petiole development was observed only in the H_2SO_4 + GA_3 (200 ppm) + KNO_3 and H_2SO_4 + GA_3 (500 ppm) + KNO_3 treatments, with mean lengths of 7.10 and 7.47 cm, respectively. The observed increases in leaf area and leaf length suggest improved photosynthetic capacity and enhanced aerial tissue development under this treatment.

Root traits were also strongly influenced by the priming treatments. The H_2SO_4 + GA_3 500 ppm + KNO_3 treatment yielded the highest root volume (26.47 cm^3), root length (23.23 cm), and root fresh weight (2.70 g) (Fig .2), indicating a substantial improvement in root system development. Although the H_2SO_4 + GA_3 (200 ppm) treatment showed a numerically higher root dry weight (0.35 g), differences among treatments were not statistically significant for this trait ($P = 0.078$; Table 2).

The greater root volume and length observed under the H_2SO_4 + GA_3 (500 ppm) + KNO_3 treatment were accompanied by the highest shoot dry weight (1.71 g). These results indicate that this treatment promoted several aspects of early seedling growth, particularly root development and shoot dry matter accumulation.

Table 2. ANOVA results (mean squares) for the response of germination and seedling growth traits of *Cycas revoluta* to various priming treatments.

S. O. V	DF	Root traits			
		Root Length	Root Dry Weight	Root Fresh Weight	Root Volume
Treatment	4	79.818**	0.019 ns	2.225**	11.181**
Error	10	0.5307	0.0065	0.1292	0.4693

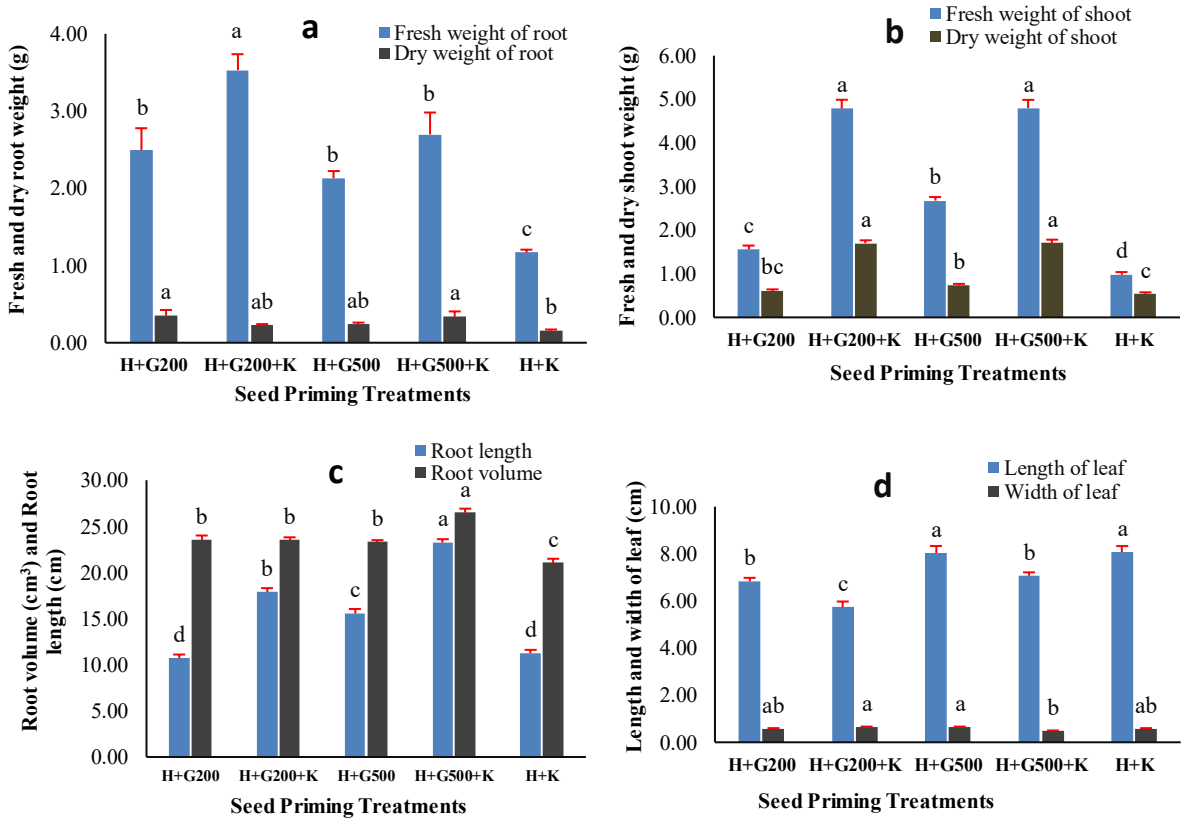
Continued

S. O. V	DF	Shoot traits			Physiological traits		
		Shoot Dry Weight	Shoot Fresh Weight	Plant Height	Germination Percentage	Hypocotyl Diameter	Chlorophyll Content Index (CCI)
Treatment	4	1.056**	9.515**	40.129**	2053.73**	0.172**	0.181 ns
Error	10	0.0082	0.0566	1.173	14.07	0.018	0.1234

Continued

S. O. V	DF	Leaf traits					
		Petiole Length 1	Petiole Length 2	Leaf Length	Leaf Width	Number of Leaves	Leaf Area
Treatment	4	4.249**	47.793**	2.80 **	0.014 ns	0.900**	634.64**
Error	10	0.284	0.0467	0.153	0.0033	0.00012	5.339

*, ** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively; ns = non-significant.



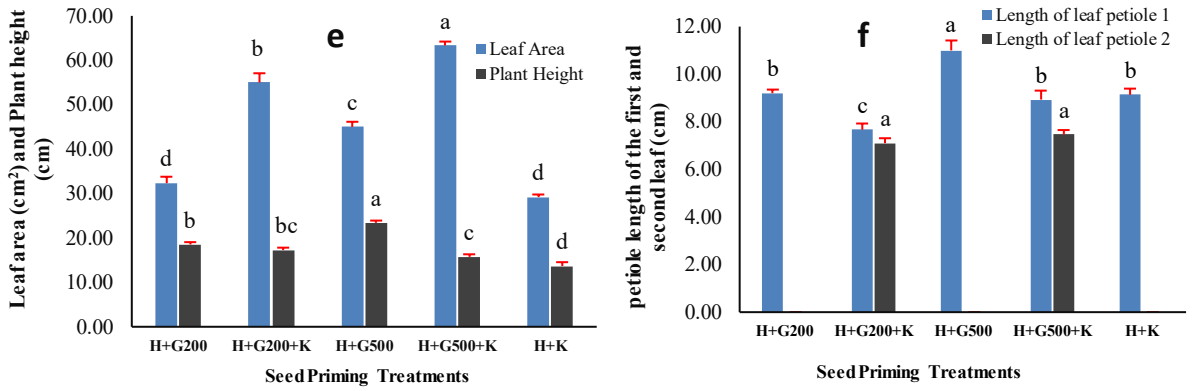


Figure 2. Effects of seed priming treatments on the morphological growth characteristics of *Cycas revoluta* seedlings, including fresh and dry root weight (a), fresh and dry shoot weight (b), root length and root volume (c), Length and width of leaf (d), leaf area and plant height (e), and petiole length of the first and second leaves (f). Treatments were H+G200 (H_2SO_4 + GA_3, 200 ppm), H+G200+K (H_2SO_4 + GA_3, 200 ppm + KNO_3), H+G500 (H_2SO_4 + GA_3, 500 ppm), H+G500+K (H_2SO_4 + GA_3, 500 ppm + KNO_3), and H+K (H_2SO_4 + KNO_3). Values are presented as mean ± SE. Different letters indicate significant differences among treatments according to the LSD test at $P < 0.05$.

3.3. Chlorophyll Content Index (CCI)

Although the H_2SO_4 + GA_3 200 ppm treatment recorded the highest relative chlorophyll content (1.16 CCI units), the differences among treatments were not statistically significant according to ANOVA (Table 2). The absence of a significant response suggests that, despite their pronounced effects on growth and germination, the priming treatments did not substantially alter chlorophyll accumulation in the early seedling stage

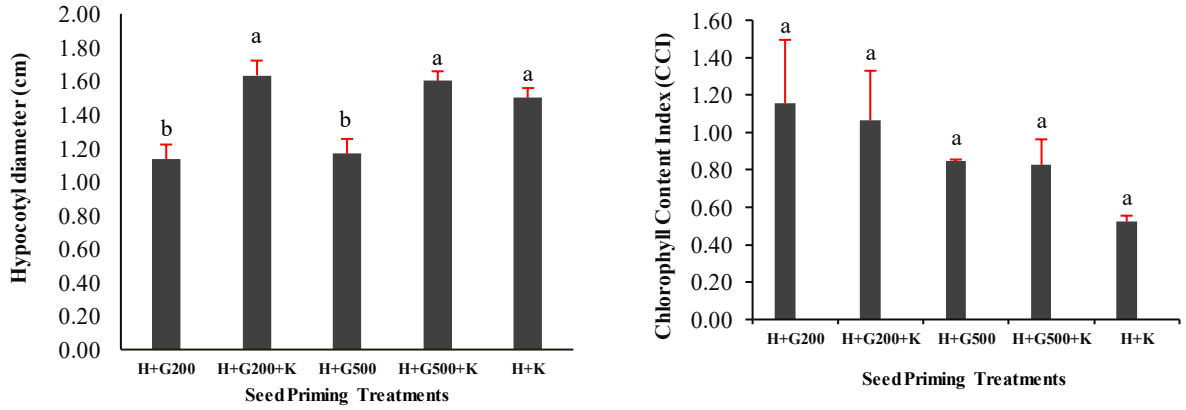


Figure 3. Effect of seed priming treatments on Chlorophyll Content Index (CCI) and Hypocotyl diameter of *Cycas revoluta*. Treatments were: H+G200 (H_2SO_4 + GA_3 at 200 ppm), H+G200+K (H_2SO_4 + GA_3 at 200 ppm + KNO_3), H+G500 (H_2SO_4 + GA_3 at 500 ppm), H+G500+K (H_2SO_4 + GA_3 at 500 ppm + KNO_3), and H+K (H_2SO_4 + KNO_3). Values are presented as mean ± SE. Different letters indicate significant differences among treatments according to the LSD test at $P < 0.05$.

4. Discussion

4.1. Germination Response to Dormancy-Breaking Treatments

The present study demonstrated that the combined treatment of H_2SO_4 (15 M), GA_3 (500 ppm), and KNO_3 (3%) was the most effective approach among the treatments evaluated for improving seed germination in *Cycas revoluta*, resulting in a germination percentage of 86.67% within approximately three months. This response may reflect the contribution of multiple treatment components to germination improvement; however, the specific physiological mechanisms underlying this response were not directly investigated in the present study. No germination was recorded in the untreated and H_2SO_4-only controls during the three-month observation period, whereas the other combined treatments exhibited different levels of germination success. However, the viability of non-germinated seeds was not assessed at the end of the experiment. Therefore, the absence of germination cannot necessarily be interpreted as evidence of seed death or permanent dormancy.

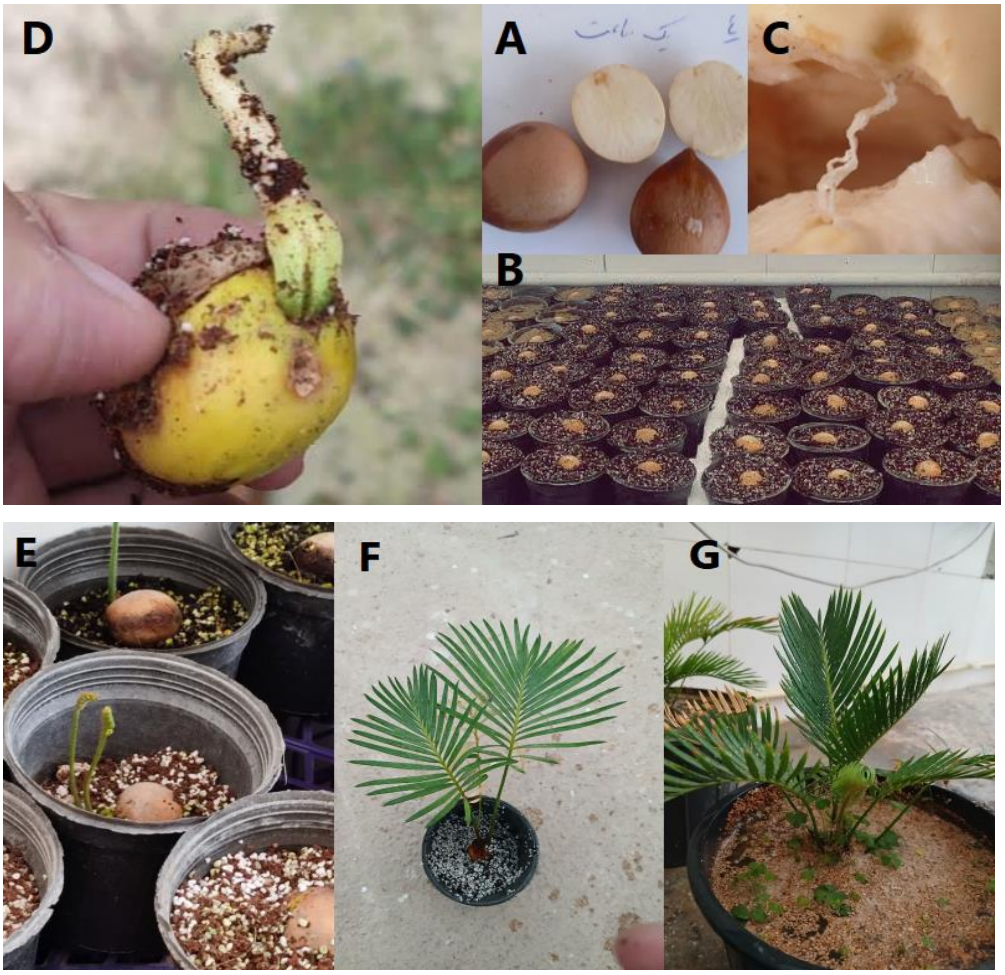


Figure 4. Sequential stages of *Cycas revoluta* seed preparation, germination, and seedling development under priming treatments. Sulfuric acid–scarified seeds showing visual observation of embryo integrity following seed coat removal (A), followed by sowing of primed seeds in a well-drained substrate (B). Early internal embryo development observed after seed dissection several weeks after sowing is shown (C), followed by radicle elongation and initial seedling establishment (D). Young seedlings subsequently developed their first emerging leaves (E), which expanded into pinnate juvenile foliage (F), ultimately developing into two-year-old nursery-grown plants exhibiting characteristic cycas morphology (G).

The poor germination performance of the H_2SO_4 + KNO_3 treatment, relative to the GA_3-containing treatments, suggests that sulfuric acid scarification and nitrate supplementation alone were insufficient to achieve the germination response observed in treatments containing GA_3. This finding aligns with previous studies reporting prolonged dormancy and delayed germination in *Cycas* seeds (Dehgan and Schutzman, 1989; Qin *et al*., 2023). Sulfuric acid scarification may have contributed to germination by modifying the seed coat; however, because seed coat permeability, water uptake, and oxygen diffusion were not measured, these processes cannot be confirmed as the mechanisms responsible for the observed response. The relatively low germination percentage observed in the H_2SO_4 + KNO_3 treatment also suggests that scarification and KNO_3 application alone were insufficient to produce germination levels comparable to those obtained with GA_3-containing treatments.

The addition of GA_3 markedly enhanced germination, particularly at 500 ppm. Gibberellins are known to promote dormancy release by stimulating embryo growth, enhancing reserve mobilization, and inducing the synthesis of hydrolytic enzymes necessary for germination. Furthermore, exogenous GA_3 can counteract the inhibitory effects of abscisic acid (ABA), thereby shifting the hormonal balance toward germination (Lima and Oliveira, 2024). In the present study, however, endogenous ABA and GA levels, enzyme activities, and reserve mobilization were not measured. Thus, these processes represent possible explanations based on previous literature rather than mechanisms demonstrated experimentally in *C. revoluta*. The superior performance of the treatment containing 500 ppm GA_3 indicates that this treatment regime was more effective under the conditions tested. It should be noted that GA_3 concentration and soaking duration were varied simultaneously (200 ppm for 48 h versus 500 ppm for 24 h). Consequently, their individual effects cannot be separated, and the higher germination response observed under the 500 ppm regime cannot be attributed solely to GA_3 concentration. Future research employing

a factorial combination of GA_3 concentrations and exposure durations is warranted to determine their independent and interactive effects.

The further improvement obtained by incorporating KNO_3 into the treatment may be explained by the dual role of nitrate as both a nutrient and a signaling molecule. Nitrate has been reported to stimulate seed germination through interactions with hormonal signaling pathways and by enhancing respiratory and metabolic activities associated with embryo growth (Yan and Chen, 2020). Nevertheless, these physiological responses were not measured in the present study. The higher germination percentage obtained with the combined H_2SO_4 + GA_3 + KNO_3 treatment indicates that this combination was more effective than the other tested treatments, but the individual contribution of each component and the mechanisms underlying their combined effects require further investigation.

These findings are consistent with those of Zarchini *et al*. (2011), who reported enhanced germination of *C. revoluta* following sulfuric acid and hot-water treatments. Although the germination response in the present study was higher than that reported in their study, differences in experimental conditions and treatment protocols should be considered when comparing the results. Collectively, the findings indicate that the combined treatment evaluated here was the most effective among the tested treatments for improving germination under the experimental conditions.

4.2. Root Development

The significant increase in root volume (26.47 cm^3) observed under the H_2SO_4 + GA_3 (500 ppm) + KNO_3 treatment indicates that the effects of the treatments were also evident in certain aspects of early seedling growth. Root system development is a key determinant of seedling establishment because it contributes to water uptake, nutrient acquisition, and anchorage (Taiz *et al*., 2015). The greater root volume observed in the present study represents a measurable difference in root growth; however, the physiological processes responsible for this response were not investigated.

In addition, GA_3 has been reported to promote cell division and elongation, thereby contributing to plant growth (Lima and Oliveira, 2024). The nitrate supplied by KNO_3 may also support plant growth through its role in nitrogen nutrition and metabolism. Similar positive effects of nitrate on root development have been documented in numerous plant species (Yang *et al*., 2025). These findings provide possible explanations for the observed differences; nevertheless, cell division, nitrogen assimilation, and nutrient uptake were not directly measured in this study. Therefore, the greater root volume should be interpreted as an observed growth response rather than evidence of a specific underlying physiological mechanism.

4.3. Shoot Growth and Leaf Development

The increase in leaf area to 63.33 cm^2 under the most effective treatment indicates that the treatment-related differences extended to leaf development during early seedling growth. Leaf area is an important morphological trait associated with light interception and potential photosynthetic performance (He *et al*., 2025). However, because gas exchange, chlorophyll fluorescence, and other direct indicators of photosynthetic activity were not measured, the increased leaf area cannot be interpreted as evidence of enhanced photosynthetic capacity. The enhanced leaf expansion observed in the present study may reflect differences in early seedling growth among treatments.

Furthermore, the observed differences in root and shoot growth may be related to the overall growth responses of seedlings to the applied treatments. However, water and nutrient uptake, biomass allocation, and source–sink relationships were not evaluated. Similar relationships between germination treatments and subsequent seedling growth have been reported in other species (Yan *et al*., 2022). Accordingly, the present findings demonstrate improvements in selected morphological traits but do not establish the physiological processes responsible for these responses.

4.4. Chlorophyll Content Index (CCI)

Although seedlings treated with GA_3 at 200 ppm exhibited numerically higher relative chlorophyll content (CCI index), differences among treatments were not statistically significant. This finding suggests that leaf chlorophyll status in *Cycas revoluta* seedlings was less responsive to dormancy-breaking treatments than growth-related traits such as germination percentage, root volume, and leaf area. Similar observations have been reported in other species, where seed treatments substantially influenced seedling growth and establishment but had limited effects on chlorophyll accumulation during the early stages of development (Cardarelli *et al*., 2022).

The absence of significant differences in CCI indicates that the applied treatments did not produce statistically detectable changes in this chlorophyll-related index under the conditions of the present study. However, CCI measurements provide an indirect estimate of leaf chlorophyll status and cannot independently establish

photosynthetic capacity or leaf nitrogen status. Therefore, the differences in germination and seedling growth observed among treatments were not accompanied by statistically significant differences in CCI.

Finally, it should be acknowledged as a study limitation that this evaluation was carried out as a single trial (2023–2024). Although the greenhouse environment was tightly regulated to maintain consistent diurnal thermal conditions (30–35°C Day / 25–27°C night) regardless of external seasonal fluctuations, intrinsic biological factors, such as mother plant background, harvest timing, and seed-lot dormancy depth, can inherently influence germination kinetics. Repeating the experiment over multiple independent years or with distinct seed cohorts under the same controlled regimes would further strengthen statistical robustness and validate the broad applicability of these priming protocols.

4.5 Study limitations and future perspectives

The physiological mechanisms potentially underlying the improved germination response observed in this study were not directly investigated. In particular, seed water uptake, seed coat permeability, endogenous ABA and GA levels, α-amylase activity, starch mobilization, photosynthetic capacity, and oxygen availability within the seed or embryo were not measured. Therefore, the proposed involvement of these processes should be considered as possible explanations based on previous literature rather than experimentally confirmed mechanisms. Furthermore, the assessment of embryo damage was based solely on visual inspection, without tetrazolium (TZ) viability testing or histological examination. Consequently, the absence of visible damage does not necessarily confirm embryo viability or internal tissue integrity. In addition, the effects of different H_2SO_4 concentrations and exposure durations were not evaluated; therefore, the potential risks associated with the applied treatment (15 M for 30 min) and the optimal treatment conditions remain to be investigated. Future studies incorporating physiological, biochemical, and histological assessments, together with acid-treatment dose–response experiments, are needed to verify these potential mechanisms and provide a more comprehensive understanding of dormancy release in *Cycas revoluta*.

5. Conclusion

The results of this study demonstrate that combined chemical and hormonal priming with H_2SO_4 (15 M), GA_3 (500 ppm), and KNO_3 (3%) substantially improved seed germination and several aspects of early seedling growth in *Cycas revoluta* under the tested conditions and in the perlite–cocopeat substrate. The improvements were particularly evident in root development and leaf expansion, whereas the Chlorophyll Content Index (CCI) was not significantly affected by the treatments. These findings indicate that the combined treatment was the most effective among those evaluated for improving germination and early seedling growth. However, further studies under different environmental and nursery conditions are required to assess its reproducibility and practical applicability. Moreover, because GA_3 concentration and soaking duration were varied simultaneously, their individual contributions to the observed responses cannot be distinguished.

References

Bakhshy, E., Zarinkamar, F., Nazari, M., and Zare, Z. 2026. Key signaling pathways regulating gene expression during seed germination. *Critical Reviews in Plant Sciences*, 1–26.

Benjelloun, J., Bouzroud, S., Triqui, Z.E.A., Alami, Q.L., Layachi, R., Smouni, A., and Guedira, A. 2021. Warm stratification improves embryos development and seed germination of *Cycas revoluta*. *Advances in Horticultural Science*, 35(1): 91–96.

Calonje, M., Kay, J., and Griffith, M.P. 2011. Propagation of cycad collections from seed: applied reproductive biology for conservation. *Sibbaldia: The International Journal of Botanic Garden Horticulture*, 9: 79–96.

Cardarelli, M., Woo, S.L., Rouphael, Y., and Colla, G. 2022. Seed treatments with microorganisms can have a biostimulant effect by influencing germination and seedling growth of crops. *Plants*, 11(3): 259.

Corbineau, F. 2022. Oxygen, a key signalling factor in the control of seed germination and dormancy. *Seed Science Research*, 32(3): 126–136.

Dehgan, B., and Schutzman, B. 1989. Embryo development and germination of *Cycas* seeds. *Journal of the American Society for Horticultural Science*, 114(1): 125–129.

Duermeyer, L., Khodapanahi, E., Yan, D., Krapp, A., Rothstein, S.J., and Nambara, E. 2018. Regulation of seed dormancy and germination by nitrate. *Seed Science Research*, 28(3): 150–157.

Gholamzadeh, A.A., Mousavi-Fard, S., and Rezaei Nejad, A. 2022. Morphological and physiological characteristics for evaluation of salicylic acid effects on *Celosia argentea* L. under salinity stress. *Iranian Journal of Plant Physiology*, 12(1): 4027–4037.

Gonçalo, R.R., de Souza Palma, R.M., Gonzaga, D.R., de Almeida, O.J.G., and Kolb, R.M. 2026. Higher temperatures and lower osmotic potentials negatively impact *Rhipsalis* Gaertn. (Rhipsalideae, Cactaceae) germination and seedling survival. *Plant Ecology*, 227(1): 5.

He, P., Ye, Q., Yu, K., Wang, H., Xu, H., Yin, Q., et al. 2025. Growing-season precipitation is a key driver of plant leaf area to sapwood area ratio at the global scale. *Plant, Cell and Environment*, 48(1): 746–755.

Lima, J.M.E., and Oliveira, J.A. 2024. Breaking dormancy in *Cycas revoluta*: a study of seed morphological characterization and dormancy mechanisms. *Journal of Seed Science*, 46: e202446035.

Marler, T.E. 2025. Root growth and branching of two *Cycas* species are influenced by form of nitrogen fertilizer. *Agronomy*, 15(10): 2280.

Marler, T.E. 2026. Filial effects of ephemeral cycad seedlings contribute nitrogen to the parents' rhizosphere. *Ecologies*, 7(2): 33.

Nonogaki, H. 2019. Seed germination and dormancy: the classic story, new puzzles, and evolution. *Journal of Integrative Plant Biology*, 61(5): 541–563.

Paparella, S., Araújo, S.D.S., Rossi, G., Wijayasinghe, M.A.L.A.K.A., Carbonera, D., and Balestrazzi, A. 2015. Seed priming: state of the art and new perspectives. *Plant Cell Reports*, 34(8): 1281–1293.

Qin, M.Y., Zhao, H., Zhang, S.L., Shan, X.Y., Han, Y., Feng, L.Y., Wuyun, T.N., Li, F.D., Liu, W.Y., and Zhu, G.P. 2023. Seed dormancy and germination mechanism of rare and endangered plants. *Pakistan Journal of Botany*, 55(6): 2295–2301.

Saeidi, T.S., Zahedi, B., Mousavi-Fard, S., and Ahmadpour, A. 2020. Response of germination and seedling growth of pepper cultivars to seed priming by plant growth regulators. *International Journal of Horticultural Science and Technology*, 7(1): 59–68.

Taiz, L., Zeiger, E., Møller, I.M., and Murphy, A. 2015. *Plant Physiology and Development*. 6th ed. Sinauer Associates, Sunderland, CT.

Tian, M.H., Zhao, Z.W., and Tang, A.J. 2016. Desiccation sensitivity and effects of temperature and moisture content of the substrate during storage on after-ripening in seeds of *Cycas revoluta*. *Plant Physiology Journal*, 52(2): 225–233.

Yan, A., and Chen, Z. 2020. The control of seed dormancy and germination by temperature, light and nitrate. *The Botanical Review*, 86(1): 39.

Yan, M., Zhang, L., Ren, Y., Zhang, T., Zhang, S., Li, H., et al. 2022. The higher water absorption capacity of small root system improved the yield and water use efficiency of maize. *Plants*, 11(17): 2300.

Yang, Z., Yan, H., Liu, H., Yang, L., Mi, G., and Wang, P. 2025. Enhancing crop nitrogen efficiency: the role of mixed nitrate and ammonium supply in plant growth and development. *Biology*, 14(5): 546.

Zarchini, M., Hashemabadi, D., Kaviani, B., Fallahabadi, P.R., and Negahdar, N. 2011. Improved germination conditions in *Cycas revoluta* L. by using sulfuric acid and hot water. *Plant Omics Journal*, 4(7): 350–353.