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Research article

Methods of Breaking Seed Dormancy for Four Species of Ornamental Cacti

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Abstract

The aim of this study was to investigate methods for breaking the seed dormancy of cacti that are cultivated in Thailand. To stimulate seed germination and decrease the production time for cacti, the research used four cacti species: *Astrophytum asterias*, *Echinocactus grusonii*, *Myrtillocactus geometrizans*, and *Turbinicarpus alonsoi*, which are extensively grown as ornamental cacti species in Thailand. The experimental design used in this study was a completely randomized design (CRD). The pot experiment consisted of 4 dormancy breaking methods and six replicates, 30 seeds per pot. For the control, seeds were sown onto planting media directly. In the experimental samples, seeds were subjected to different pre-soaking treatments: soaking for 24 h in room temperature water (28±2°C), GA_3 500 ppm for 24 h, and H_2SO_4 (50%) for 30 min. The seed germination and plant growth data were collected after sowing cacti seeds for two months. The results showed that soaking cacti seeds in room temperature water produced the highest germination rates for *A. asterias*, *E. grusonii*, and *M. geometrizans*. In addition, this treatment also reduced the production time of *M. geometrizans*. Concurrently, *A. asterias*, *E. grusonii*, and *M. geometrizans* presented higher stem diameters and root lengths of the seedlings, as well as the best result on the seedling vigor of cacti. Soaking seeds with GA_3 resulted in the highest germination percentage of *T. alonsoi* and accelerated seed germination of *A. Asterias*, and *T. alonsoi*. Moreover, this treatment led to the maximum root lengths and seedling vigor of *T. alonsoi*. However, soaking seeds with H_2SO_4 was a treatment that gave *E. grusonii* the maximum root length and seedling vigor.

Keywords: breaking dormancy; cactus seed; seed germinstion; GA_3; H_2SO_4; water

1. Introduction

Cacti are dicotyledonous plants that are classified in the Cactaceae family. There are more than 150 genera and 2,500 species (Slaba, 1992). Collection and severe habitat disturbances have affected some of these cacti species (Goettsch et al., 2015). People collect cacti for ornamental purposes (Ortega-Baes et al., 2010). On the IUCN Red List, 1478 species of cacti are found to be endangered (Goettsch et al., 2015). To solve these

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problems, it is necessary to study seed germination to reduce the importation of natural cacti (Flores et al., 2008). Some cactus seeds are physiologically dormant (Rojas-Aréchiga & Vázquez-Yanes, 2000). Many varieties must wait for the fruit to ripen and break seed dormancy, so cacti seeds such as *Opuntia tomentose* grow slowly (Orozco-Segovia et al., 2007). In addition, it was reported that the imbibing process was an important factor affecting seed dormancy for *Melocactus curvispinus* (Arias & Lemus, 1984) and *Stenocereus thurberi* (Zimmer, 1969). Flores et al. (2008) also found that storing *Turbinicarpus lophophoroides* and *Turbinicarpus pseudopectinathus* seed for four years could increase germination. In addition, it was observed that thick seed coats caused seed to remain dormant for a long time (Olvera-Carrillo et al., 2003), and phenol compounds present in seed testa could prevent seed germination (Debeaujon et al., 2000).

Cactus seeds generally have a low germination rate (Rojas-Aréchiga & Vázquez-Yanes, 2000). Therefore, it is necessary to stimulate seed germination before planting. Breaking seed dormancy could be done in many ways such as soaking seeds with growth regulators (GA_3 and IAA) (Hillhorst & Karssen, 1992; Iglesias & Babiano, 2006), water, acid, and 1N HNO_3 solution for 24 h and drying the seeds at 50°C (International Seed Testing Association, 2010). The seed germination process can be successful if water was imbibed through the seed coat (Woodstock, 1988). In the first phase, water imbibing occurs rapidly and is essential for starting seed germination (Harb, 2013). The second phase is rehydration; water is used to stimulate the synthesis of enzymes and precursors in the process (Bewley & Black, 1982). Therefore, if seeds cannot absorb enough water from soil or planting material, the seeds tend to die. Because of this, before sowing seeds on planting material, the seeds should be soaked in water to improve the speed of imbibition process and thus germination (Schmidt, 2000). Monteon-Ojeda et al. (2021) found that soaking *Stenocereus zopilotensis* seeds with water for 6 h and *O. lindheimeri* seeds for 9 h presented the highest germination rate at 48.6% and 45%, respectively (Pilcher, 1970). H_2SO_4 was used to break seed dormancy. Soaking seeds with H_2SO_4 eliminated blockage at the micropyle (water gap) and hilum of the seeds (Wu et al., 2023), increasing temperature and stimulating metabolic signals in the germination process. For example, soaking *O. discuss* seeds with H_2SO_4 for 30 min gave the highest germination rate at 83%, in contrast with *O. lindheimeri*, which had a germination rate of 34% (Potter et al., 1984). Gibberellins are one of the phyto regulators that are synthesized in plant tissues. An important role of gibberellic acid (GA_3) is to stimulate the synthesis of α-amylase, an enzyme that converts starch in seed to sugar during the germination process (Tigabu & Oden, 2001). The activity of α-amylase primarily occurs during seed development, and its activity gradually decreases when plants develop into mature stage (Helland et al., 2002). GA_3 is another plant growth regulator that can be used for breaking seed germination (Lima & Meiada, 2017). High concentrations of GA_3 could inhibit the germination rate or increase the germination rate of some cacti species (Rojas-Aréchiga & Vázquez-Yanes, 2000). This may be related to the different sensitivity of seed tissue to GA_3 (Kucera et al., 2005). Rojas-Aréchiga et al. (2011) reported that growing *Opuntia rastrera* seeds on agar with application of GA_3 at 1000 ppm produced a high germination rate of 40%. GA_3 at 25 ppm in MS formula applied to *Opuntia* sp. (*O. amyclaea*, *O. ficus-indica*, *O. albicarpa*, *O. megacantha,* and *O. streptacantha*) promoted high a germination percentage at 90% (Tigabu & Oden, 2001).

Astrophytum asterias, *Echinocactus grusonii*, *Myrtillocactus geometrizans*, and *Turbinicarpus alonsoi* are commonly grown as ornamental cacti in Thailand, but in their native habitat, they are close to extinction (Ochoa et al., 2015). For cacti, seed dormancy occurs in two patterns, i.e. physical dormancy and physiological dormancy (Rojas-Aréchiga & García-Morales, 2022). Many reports revealed that the seeds of some cacti such as

Echinocactus platyacanthus and *Astrophytum capricorne* displayed physical dormancy (Rojas-Aréchiga & Vázquez-Yanes, 2000; Ochoa et al., 2015), whereas the seeds of others such as *Astrophytum myriostima*, *Turbinicarpus lophophoroides,* and *Turbinicarpus pseudopectinatus* demonstrated physiological dormancy (Rojas-Aréchiga & Vázquez-Yanes, 2000; Flores et al., 2008). However, some aspects of these report about the dormancy of these four cacti species remain unclear. There were few reports related to the germination rate of the four cacti species and the methods that could be used to improve those rates. Therefore, this study investigated effective strategies for breaking seed dormancy in the four cacti species to produce strong seedlings with high germination rates and lower production.

2. Materials and Methods

2.1 Seed collection

Seeds of four cacti species were collected from different areas. *Astrophytum asterias*, *Echinocactus grusonii*, and *Turbinicarpus alonsoi* were collected from a cactus greenhouse in Chiang Mai, Thailand. At the same time, *Myrtillocactus geometrizans* seeds were collected from the planting areas located between San Felipe Guanajuato and San Luis de la Paz Guanajuato, Mexico. The seeds of all species were collected from May to June 2023.

2.2 Seed testing before the experiment

2.2.1 Imbibition test

In this experiment, 100 cacti seeds of each species were weighed and placed in Petri dishes. The seeds were soaked with distilled water (50 mL per dish) and then weighed again to find the maximum imbibition weight. The data was used to calculate an imbibition percent (IP) according to the Lima and Meiado (2017) formula.

$$IP = (\text{maximum imbibition weight} - \text{seed dry weight}) \times 100$$

2.2.2 Tetrazolium test for seed viability

Seeds from 4 cactus species were divided into three replicates, 50 seeds per replicate. The seeds were soaked in distilled water for 24 h and then cut in half at the middle position. Embryos were soaked in 0.6% tetrazolium solution for 16 h at 30^{o}C (ISTA, 2003) before being observed under a stereo microscope to check the percentage of embryo staining. In this experiment, if the seeds presented a coloration of more than 50%, they were considered viable seeds.

2.3 Study area and media preparation

All experiments were conducted under 50% shaded in greenhouse conditions with light intensity 1490 µmol/m^2/s from August to October 2023 at King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok, Thailand. Planting material consisted of peat moss and pumice stone, size No. 00, in 1:1 proportion. Each 2-inch pot was filled with 150 mL of the planting material in the bottom, 1 g of slow-release fertilizer (13-13-13 osmocote),

and some granular insecticides (Dinotefuran). A fungicide-mixed solution was prepared by diluting 1.5 g of fungicide (Captan) with 1 L of water. The solution was then poured onto the planting material surface until it reached the planting pot bottom, and the cacti seeds were then sown.

2.4 Seed germination experiment

This experiment was planned as a completely randomized design (CRD) with six replicates and 30 seeds per replication. The four methods for breaking dormancy were as follows: T1) seeds were sown onto the planting material directly (control), T2) seeds were soaked in room temperature water (28±2°C) for 24 h, T3) seeds were soaked in 500 ppm GA_3 (Gibberellic acid) for 24 h, and T4) seeds were soaked in 50% H_2SO_4 for 30 min, For T2-T4 treatments, seeds were cleaned by rinsing with water for 3 min before sowing on the planting pot.

2.5 α -amylase assay

On the 1st and 7th days after sowing, the cacti seeds were picked up and dried in a hot air oven at 60°C before being stored in a desiccant. The testing of α-amylase activity was performed following Bernfeld's technique with some modifications (Bernfeld, 1955). Each species of cacti seeds was weighed at 0.05 g in a centrifuge tube, and 8 mL of Tris buffer was then shaken with a shaker for 60 min. Then, it was filtered with filter paper and increased volume to 8 mL using tris buffer. The solution was stored at -20°C for analysis of amylase enzyme. Reducing sugar (RS) was measured by hydrolyzing soluble starch with 3,5-dinitro salicylic acid (DNS reagent). The absorbance was then measured using a spectrophotometer at a wavelength of 540 nm.

2.6 Plant growth analysis and statistical analysis

All growth parameters were collected at 30 and 60 days after sowing seeds in the planting pots. The plant growth parameters studied included stem diameter, stem length, and root length. Plant growth parameters were measured. The germination percentage (GP) was calculated using the Kader formula (2005): GP = (number of normal seedlings that germinate/ number of seeds sown) × 100 (Kader, 2005). The seeding vigor index (SVI) was calculated by adapting the Al-Ansari and Ksiksi formula: SVI = GP × (root length + stem diameter) (Al-Ansari & Ksiksi, 2016). The mean germination time (MGT) was calculated from Ellis and Roberts formula: MGT = ∑(n×d) / N; where n = the number of normal seedlings each day, d = number of days since the start of the test, and N = total number of seeds germinated at the end of the test (Ellis & Roberts, 1980).

All collected data were analyzed using completely randomized designs (CRD) and applied with 10 statistix programs. The means of this experiment was compared using the least significant difference test ($p<0.05$).

3. Results and Discussion

3.1 Seed imbibition and seed viability test

The results indicated that all treatments of the 4 cacti species had different water imbibability and seed viability percentages. The tetrazolium test showed that *A. asterias*

seeds had a 63.33% mean viability (Table 1). The imbibition percentage of *E. grusonii* seeds was 0.017%. Moreover, *E. grusonii* seeds treated with TZ stain indicated that the dye was thoroughly coated around embryos and had a viability percentage at a moderate level of 47.06%. The imbibition percentage of *M. geometrizans* seeds was 0.025%. Also, treating *M. geometrizans* seeds with TZ stain produced a light dye staining on the embryo's skin and a viability percentage of 53.49%. From this experiment, *T. alonsoi* seeds had an imbibition percentage of 0.015% and also gave a great result on TZ staining, presenting with proper coverage of red dye on the embryo's skin. Moreover, this species had the highest percentage of cell viability, approximately 84.00%.

Table 1. Imbibition percentage and seed viability percentage in the seeds of four cacti

Species	Imbibition Percentage (%)	Viability Percentage (%)	Staining from TZ Staining of Embryo
Astrophytum asterias	0.456	63.33	
Echinocactus grusonii	0.017	47.06	
Myrtillocactus geometrizans	0.025	53.49	
Turbinicarpus alonsoi	0.015	84.00	

3.2 Germination of cacti seeds

After using various methods to break the dormancy of *A. asterias* seeds for one day, the control had the highest α-amylase activity (0.33 μmole/mL). However, this was the same as the result for water soaking. The results also indicated that soaking *A. asterias* seeds in water for 7 days produced the highest level of α-amylase activity while soaking seeds in H_2SO_4 produced the lowest α-amylase activity (Figure 1A). However, the germination percentages of all treatments were not significantly different at 7 days after sowing (DAS). From this experiment, soaking seeds in GA_3 and water achieved ~~to~~ increase germination percentage (92.67% and 92.23%, respectively) when compared with seeds soaked in H_2SO_4 (84.00%) (Figure 1B).

At 1 DAS, *E. grusonii* seeds were analyzed for a suitable dormancy-breaking method, and it was found that all treatments were statistically different with seed soaking in water, H_2SO_4, and control had the highest α-amylase activity (0.55, 0.51 and 0.50 μmole/mL, respectively). However, soaking seeds in GA_3 had the lowest α-amylase activity at 0.39 μmole/mL (Figure 1C). At 7 DAS, there were no statistical differences in α-amylase activity from all treatments, with the range of 0.63-0.67 μmole/mL. Moreover, the results indicated that, at 7 DAS, soaking in GA_3 gave the highest germination percentage (64.50%), while control showed the lowest germination percentage (46.17%). By 14 DAS, the germination percentages of all treatments were increased; however, there were no statistical differences between treatments. On the contrary, *E. grusonii* seeds soaked with H_2SO_4 and the control had the lowest percentages of germination (85.83%) at 21 and 28 DAS (Figure 1D).

For *M. geometrizans*, the result showed that α–amylase activity had no statistical differences at 1 DAS, averaging 0.21-0.23 µmole/mL (Figure 1E). Considering the results at 7 DAS, the seeds soaked with water had the highest α–amylase activity at 0.29 µmole/mL and the highest germination rate at 30.50%. In the case of H_2SO_4 soaked seeds and control, both treatments showed the lowest germination percentages (11.17% and 11.67%, respectively). At 21 and 28 DAS, the germination rate had increased between 62.33% and 70.50%. However, the data did not show any statistically significant differences (Figure 1F).

For *T. alonsoi*, at 1 DAS, seeds soaked with H_2SO_4 showed the significantly highest α-amylase activity of 0.45 µmole/mL (Figure 1G). While for the control, water, and GA_3 soaked seeds gave lower α-amylase activity of 0.20, 0.25, and 0.27 µmole/mL, respectively. However, there were no statistical differences in α-amylase activity at 7 DAS, with values in the range of 0.21-0.31 µmole/mL. Seed soaked with GA_3 gave the highest germination percentage (43.83%), and the percentage was increased by 21 DAS (61.17-70.00%), while soaking seeds with H_2SO_4 produced the lowest germination rate (15.50%) at 7 DAS. However, at 28 DAS, the germination percentages were not significantly different (Figure 1H).

3.3 Growth of four cacti species after breaking seed dormancy for 30 days after sowing

After soaking *A. asterias* seeds in GA_3, it was found that the seeds had an average mean germination time (MGT) of 3.88±0.16 days, which was faster than the germination times of other treatments (Table 2). After breaking dormancy for 30 days, the seedlings from water soaked seeds had the largest stem diameter (5.03±0.10 mm) whereas seedlings from GA_3 soaked seeds had the lowest (4.72±0.08 mm). However, the result was reversed for seedling height. GA_3 soaked seeds germinated into the tallest seedlings with the height of 13.50±0.55 mm whereas water soaked seeds grew into the shortest with seedling height of 9.33±0.52. Growth of *E. grusonii* seeds from GA_3 soaking had the lowest germination time, averaging 7.41±1.22 days (Table 2). Soaking seeds with GA_3 also produced the largest stem diameters (4.07±0.05 mm) and plant height (14.50±0.55 mm). However, seedlings from control, water soaking, and H_2SO_4 soaking presented with nearly the same stem diameters, which were 3.88±0.04, 3.90±0.09, and 3.92±0.09 mm, respectively. Soaking *E. grusonii* seeds with water resulted in the lowest plant height (11.50±0.55 mm). In *M. geometrizans*, seedlings prepared with water soaking germinated faster than GA_3 soaking whereas there were no significant differences between parameters of stem diameter and plant height in all treatments, with the averages of 3.03±0.05-3.07±0.05 and 7.67±0.52-8.33±0.52 mm, respectively (Table 2). In *T. alonsoi*, GA_3 soaking had a strong effect on stimulating seed germination. It was indicated that this treatment had the lowest germinating time of 7.59±2.77 days (Table 2). Nevertheless, GA_3 soaking produced the smallest stem diameter at 1.75±0.05 mm. This treatment also gave the highest plant height at 8.33±0.52 mm.

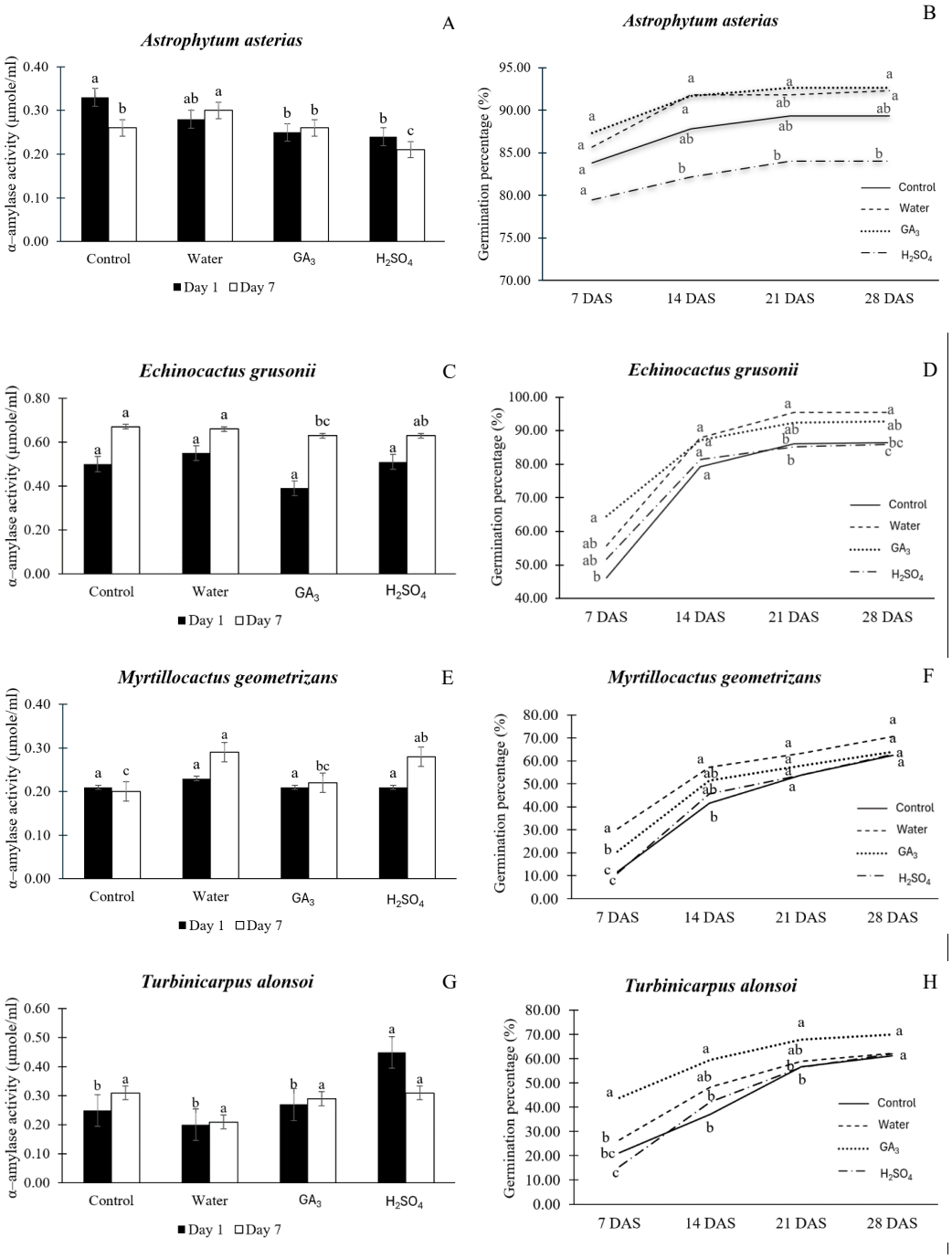

Figure 1. The α-amylase activity and percentage of cacti seed germination after sowing in *A. asterias* (A, B), *E. grusonii* (C, D), *M. geometrizans* (E, F), and *T. alonsoi* (G, H)

Table 2. Growth of four cacti species after breaking seed dormancy germination for 30 days after sowing

Treatment	Mean Germination Time (days)	Stem Diameter (mm)	Plant Height (mm)
	Astrophytum asterias		
Control	4.70±0.29^{a}	4.93±0.05^{b}	9.83±0.41^{bc}
Water	4.14±0.35^{bc}	5.03±0.10^{a}	9.33±0.52^{c}
GA_3	3.88±0.16^{c}	4.72±0.08^{c}	13.50±0.55^{a}
H_2SO_4	4.41±0.43^{ab}	4.97±0.08^{ab}	10.00±0.00^{b}
CV (%)	7.36	1.63	4.01
$LSD_{0.05}$	0.38	0.10	0.52
	Echinocactus grusonii		
Control	8.72±0.87^{a}	3.88±0.04^{b}	12.50±0.55^{b}
Water	8.10±0.91^{ab}	3.90±0.09^{b}	11.50±0.55^{c}
GA_3	7.41±1.22^{b}	4.07±0.05^{a}	14.50±0.55^{a}
H_2SO_4	7.89±0.55^{ab}	3.92±0.09^{b}	12.83±0.45^{b}
CV (%)	11.49	1.88	4.02
$LSD_{0.05}$	1.11	0.09	0.62
	Myrtillocactus geometrizans		
Control	12.21±0.71^{a}	3.03±0.05	7.83±0.75
Water	9.46±1.96^{b}	3.07±0.05	7.67±0.52
GA_3	9.72±1.54^{b}	3.07±0.05	8.33±0.52
H_2SO_4	11.83±1.41^{a}	3.05±0.05	7.67±0.55
CV(%)	13.81	1.72	7.42
$LSD_{0.05}$	1.80	ns	ns
	Turbinicarpus alonsoi		
Control	11.72±2.24^{a}	2.22±0.10^{a}	6.33±0.52^{b}
Water	9.80±1.43^{ab}	2.15±0.10^{a}	6.17±0.41^{b}
GA_3	7.59±2.77^{b}	1.75±0.05^{b}	8.33±0.52^{a}
H_2SO_4	11.86±1.61^{a}	2.13±0.09^{a}	6.33±0.55^{b}
CV(%)	20.93	4.22	7.24
$LSD_{0.05}$	2.58	0.10	0.59

^{a,b,c} Different letters in the same column are statistically different at the confidence level of 95%.
ns means no significant difference.

3.4 Growth of four cacti species after breaking seed dormancy for 60 days after sowing

Seedlings of *A. asterias* under the control, H_2SO_4, and water soaking treatments had the largest stem diameter (5.57±0.05, 5.50±0.08, and 5.48±0.08 mm, respectively), while GA_3 soaking gave the lowest stem diameter of 5.20±0.06 mm. Otherwise, GA_3 soaking gave the highest plant height of 16.17±0.75 mm, and the H_2SO_4 soaking had the shortest plant height of 12.33±0.55 mm (Table 3; Figure 2). In contrast, for the root lengths, GA_3 produced the shortest root length at 4.80±0.35 mm, but water and H_2SO_4 produced significantly longer root lengths (5.86±0.67 and 5.50±0.42 mm, respectively). In terms of seedling vigor, water soaking produced seedlings of the highest vigor (1050.60±150.39). However, the result was not significantly different from the control and GA_3 soaked seeds. Meanwhile, H_2SO_4 soaking produced the least seedling vigor (843.71±208.09).

For *E. grusonii*, GA_3 gave seedlings with the lowest stem diameter (4.53±0.10 mm) but with the tallest plant height of 16.67±0.52 mm. On the contrary, other treatments produced higher stem diameters but lower plant heights than the GA_3 soaking (Table 3; Figure 2). However, there were no statistical differences in the root lengths and seedling vigor indexes. The values ranged from 8.29±1.07 – 9.31±1.30 mm and 1181.2±107.45 – 1241.8±101.21, respectively.

For *M. geometrizans*, stem diameter and root length were the greatest when using H_2SO_4 soaking (4.77±0.04 and 4.08±0.58 mm), but did not significanlty differ from water soaking and control treatment results (Table 3; Figure 2). Plant height and seedling vigor were not statistically different in the ranges of 9.67 ± 0.82 – 10.33 ± 0.52 mm and 1182.20±98.80 – 1241.80±98.76 mm, respectively.

For *T. alansoi*, even though GA_3 soaking produced the lowest stem diameter at 2.15±0.05 mm, it gave the highest plant height at 9.33±0.52 mm (Table 3; Figure 2). Root length and seedling vigor were not statistically different and were in the ranges of 1.21±0.10 – 1.35±0.17 mm and 230.65±28.12 – 246.06±39.14 mm, respectively.

3.5 Discussion

The result of this study indicated that all four types of cactus seeds were viable and able to absorb water. The seed coat of these four cacti species was not hard. In particular, *A. asterias* presented a great result in the imbibition test. Moreover, the experiment indicated that *T. alonsoi* had a higher viability percentage than other species (Table 1). However, *E. grusonii* and *M. geometrizans* presented lower imbibition rates and viability. It is possible that *E. grusonii* and *M. geometrizans* may undergo physical dormancy, which occurs at some part of the seed coat, and this prevents water from imbibing into the seed. Moreover, it was found that soaking seeds in room temperature water for 24 h resulted in the highest germination percentage for *A. asterias*, *E. grusonii* and *M. geometrizans.* Furthermore, it also resulted in the fastest germination rate for *M. geometrizans*, probably due to high α-amylase activity on 1-7 days after sowing. In conclusion, cacti seeds should have pre-treatment to improve their growth. It is likely that soaking with water for 24 h to make the seed coat softer increases the efficiency of water entering the seed coat and stimulates the germination process. Water helps to stimulate the activity of enzymes in the seed, especially α-amylase, an important precursor for seed germination (Zimmer, 1969). Soaking seeds before sowing helps them absorb water faster, resulting in faster seed germination (Olvera-Carrillo et al., 2003). In addition, it also clears out germination

Table 3. Growth of four cacti species after breaking seed dormancy germination for 60 days after sowing

Treatment	Stem Diameter (mm)	Plant Height (mm)	Root Length (mm)	Seedling Vigor Index
Astrophytum asterias				
Control	5.57±0.05^{a}	12.83±0.75^{bc}	5.33±0.28^{ab}	973.07±42.08^{ab}
Water	5.48±0.08^{a}	13.33±0.82^{b}	5.86±0.67^{a}	1050.60±150.39^{a}
GA_3	5.20±0.06^{b}	16.17±0.75^{a}	4.80±0.35^{b}	927.13±46.70^{ab}
H_2SO_4	5.50±0.08^{a}	12.33±0.55^{c}	5.50±0.42^{a}	843.71±208.09^{b}
CV(%)	1.31	5.26	10.49	13.04
$LSD_{0.05}$	0.09	0.87	0.68	148.98
Echinocactus grusonii				
Control	4.68±0.10^{a}	14.83±0.75^{b}	9.31±1.30	1208.3±105.22
Water	4.72±0.08^{a}	14.50±0.55^{b}	8.29±1.07	1241.8±101.21
GA_3	4.53±0.10^{b}	16.67±0.52^{a}	8.58±0.70	1220.3±133.08
H_2SO_4	4.77±0.05^{a}	15.00±0.71^{b}	9.00±0.34	1181.2±107.45
CV(%)	1.81	4.06	10.54	9.31
$LSD_{0.05}$	0.10	0.75	ns	ns
Myrtillocatus geometrizans				
Control	4.68±0.08^{a}	9.83±0.75	3.63±0.48^{a}	1208.30±25.80
Water	4.72±0.05^{a}	9.67±0.82	3.77±0.46^{a}	1241.80±98.76
GA_3	4.53±0.08^{b}	10.33±0.52	3.08±0.26^{b}	1220.30±69.78
H_2SO_4	4.77±0.04^{a}	9.67±0.45	4.08±0.58^{a}	1182.20±98.80
CV(%)	1.81	6.73	12.10	9.31
$LSD_{0.05}$	0.10	ns	0.53	ns
Turbinicarpus alonsoi				
Control	2.50±0.06^{a}	7.83±0.41^{b}	1.29±0.15	232.83±44.98
Water	2.52±0.04^{a}	7.83±0.41^{b}	1.33±0.13	239.24±13.54
GA_3	2.15±0.05^{b}	9.33±0.52^{a}	1.35±0.17	246.06±39.14
H_2SO_4	2.52±0.05^{a}	7.50±0.55^{b}	1.21±0.10	230.65±28.12
CV(%)	2.47	5.84	10.74	13.96
$LSD_{0.05}$	0.07	0.57	ns	ns

^{a,b,c} Different letters in the same column are statistically different at the confidence level of 95%.
ns means no significant difference.

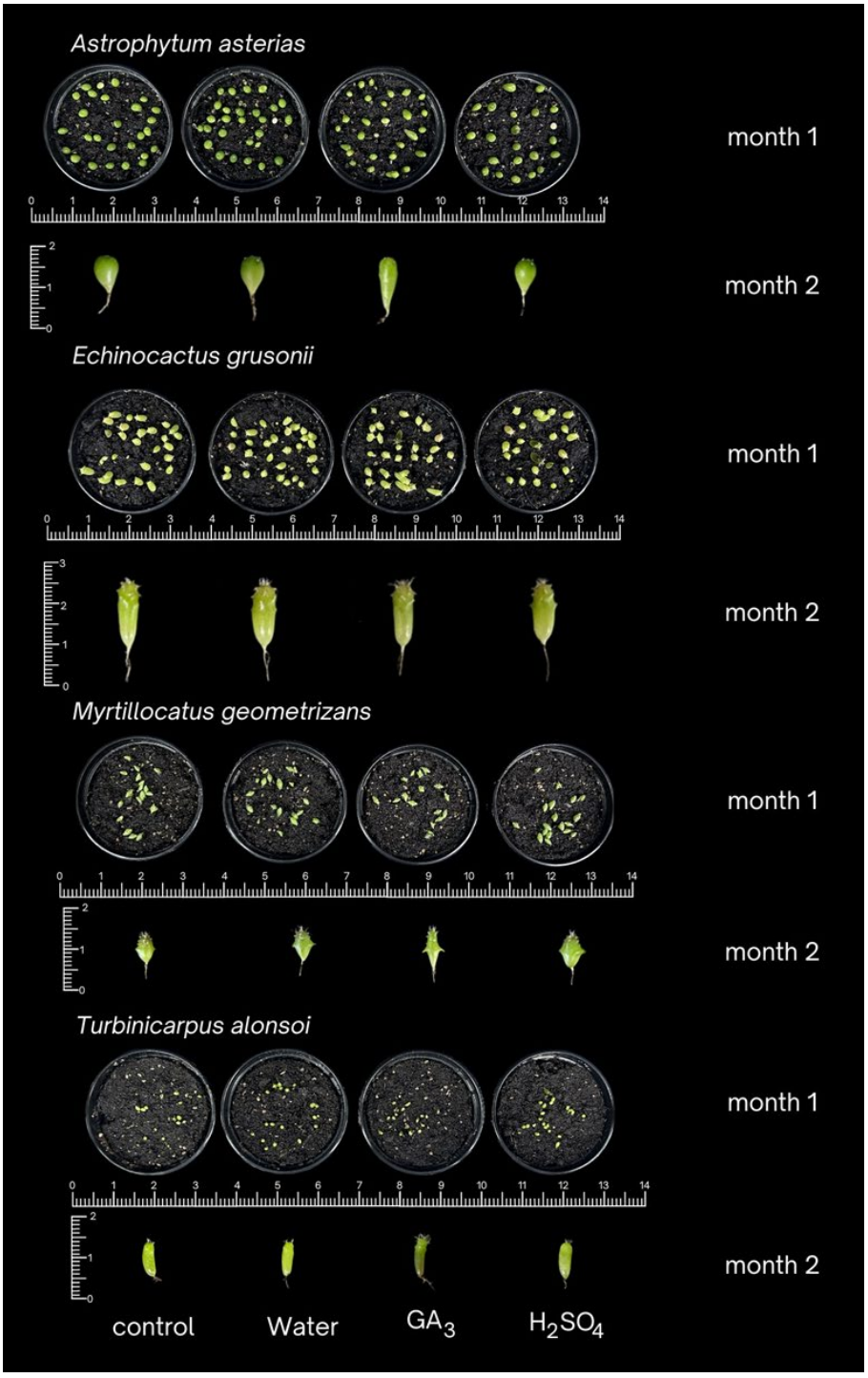

Figure 2. Growth of four cacti species using different germination-breaking dormancy methods for 1 and 2 months after sowing

inhibitors in the seed testa, resulting in an increased germination rate (Rojas-Aréchiga & Vázquez-Yanes, 2000). Presoaking with water greatly increased the growth rate, stem diameter, and root length of *A. asterias*, *E. grusonii*, and *M. geometrizans* and gave the greatest seedling vigor to the seedlings. Moreover, soaking them in water gave *T. alonsoi* seedlings the largest stem diameter. This study showed that GA_3 soaking could break the dormancy of *T. alonsoi* and *A. asterias* seeds as evidenced by *T. alonsoi* having the highest germination percentage. In addition, *A. asterias*, *E. grusonii,* and *T. alonsoi* had the fastest germination rates. The dormancy pattern could have been caused by internal factors and hormonal balance because high α-amylase activity was observed on days 1-7 after sowing. In addition, GA_3 soaking maximized the root length of *T. alonsoi*, resulting in the greatest seedling vigor. Moreover, it stimulated the maximum plant heights in all four species. This was likely because GA_3 induces the synthesis of α-amylase, which converts starch in the seeds to sugar during seed germination (Debeaujon et al., 2000) and promotes cell elongation (Mohammed, 2023). This is consistent with the study of Bárbara et al. (2015), who observed that *Melocactus azureus*, soaked in water and soaked in GA_3 for 2 h, increased the seed germination rates of 14% and 21%, respectively, compared to only 3% of seed germination in control. Soaking seeds with H_2SO_4 produced great results for all growth parameters for the cacti species, except *A. asterias*, which showed the highest stem diameter with regular sowing. Soaking *T. alonsoi* seeds with H_2SO_4 produced seedlings with a large stem diameter. Ultimately, because H_2SO_4 can make the seed coat softer, water penetrates the seeds (Rusdy, 2016), and the seed germination process occurs (Unacademy, 2023).

Therefore, it can be concluded that seeds of different cacti species responded differently to dormancy-breaking methods. Based on the mean germination time, it was found that water-soaking treatment was suitable for *M. geometrizans*, while soaking seed with GA_3 was suitable for *A. asterias*, *E. grusonii,* and *T. alonsoi*. In terms of growth, each species has a different response. From this study, water soaking was the most appropriate method for *A. asterias*, *E. grusonii*, and *M. geometrizans* based on seedling vigor. Furthermore, soaking the seeds with GA_3 seemed to be the optimal treatment for *T. alonsoi*.

4. Conclusions

The seeds of 4 cacti species responded differently to the germination stimulation methods. The most suitable method for *M. geometrizans* seeds was soaking in room temperature water for 24 h. Soaking seeds with GA_3 500 ppm for 24 h was an effective method for *A. asterias*, *E. grusonii*, and *T. alonsoi*. In terms of growth, each species had a different response as well. Soaking seeds with room temperature water was suitable for *A. asterias*, *E. grusonii,* and *M. geometrizans* while soaking seeds with GA_3 was suitable for *T. alonsoi*.

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6. Conflicts of Interest

This research has no conflict of interest.

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