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Research article

The Impact of Different Colors of Light-Emitting Diodes on Cacti Germination and Seedling Growth

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Abstract: To identify suitable light colors to break seed dormancy and enhance the growth of cactus seedlings. A short photoperiod and less light intensity affect seedlings, resulting in low germination and slow growth. Four cacti species are *A. asterias*, *E. grusonii*, *M. geometrizans*, and *T. alonsoi*, commonly grown as ornamental plants. The completely randomized design was experimental. The plant was cultured under the five different colors of light-emitting diodes (LEDs): natural light (NL), red, blue, green, and white LED, providing light for 12 hours/day. The results found that all cactus seeds can absorb water and have more than 50% viability, except for *E. grusonii*. The seed germination (%G) increased in NL and various colors of LED. White LED had the highest %G in *A. asterias*, but green LED had the highest α-amylase activity. In *E. grusonii*, *M. geometrizans*, and *T. alonsoi*, %G and α-amylase activity were the highest under red LED. In addition, all four cacti species had the lowest mean germination time under red LED. The growth of *A. asterias* and *M. geometrizans* had the highest seedling vigor, stem diameter, plant height, and root length under NL. They had the highest chlorophyll a and chlorophyll b. In *E. grusonii* and *T. alonsoi* under NL, with the highest stem diameter, plant height and increases chlorophyll a and chlorophyll b. However, white light has the maximum root length and seedling vigor. Thus, red, green, and white LEDs effectively break seed dormancy, while white LEDs stimulate cactus growth as the natural light.

Keywords: LED light; Breaking dormancy; Seed germination; Cactus growth

1. Introduction

Cacti belong to the Cactaceae family of plants. These are dicotyledonous plants. To date, 150 genera, 1,851 species, and 91 hybrids have been discovered [1]. Although countries in North America, Europe, and Asia produce millions of cacti per year, there continues to be a demand for species collected from their natural habitat. Therefore, habitat destruction and illegal mining for international trade are still dangerous for cacti [2]. Seed germination study helps conserve plants' germplasm [3]. Some cactus seeds have a low germination rate [4]. In cacti, dormancy can occur in two patterns: physiological dormancy and physical dormancy [5]. Many reports in the same genus revealed that *Echinocactus platyacanthus* and *Astrophytum capricorne* were physically dormant [4, 6]. While *Astrophytum myriostima*, *Turbinicarpus lophophoroides,* and *Turbinicarpus pseudopectinatus* were physiological dormancy [3, 4]. Furthermore, seedlings such as *Aztekium* sp., *Ariocarpus* sp., *Obregonia* sp., *Turbinicarpus* sp., etc., grow slowly

[7]. Most cacti take about one year from planting seeds until they are ready for sale. Due to their slow growth and dormant nature, cactus seeds cannot satisfy consumer demands.

Light stimulates the germination of many cacti [8]. Light (radiation) is essential for photosynthesis, photoperiod, and morphology. Solar light constitutes electromagnetic radiation ranging from 400 to 700 nm (purple, blue, green, yellow, orange, and red). Light in nature has different light qualities (wavelength) that affect plant physiology and plant metabolism differently (e.g., differences within and between cells, seed germination, seedling growth, photosynthesis, flowering, etc.), which was regulated by the species and developmental stage, or organ studied [9]. Light-emitting diodes (LEDs) have many advantages, including being small, durable, and long service life. Cold emitting temperature and options for selecting specific wavelengths to respond to different plant stages. Especially during seed germination and growth. This affects the quality of plants. In addition, it allows plants to receive consistent light, making LEDs suitable for use by plants [10]. Different wavelengths of light affect plants differently at different stages of development. For example, red light stimulates seed germination, hypocotyl elongation, and the plant's high chlorophyll pigment absorption [11]. Typically, blue light controls plants' growth and morphology, for example, in inhibiting the extensive growth of stems and seed germination. However, it promotes the potential of seed germination in the family Cucurbitaceae [12]. Phytochrome can convert to its biologically active far-red-absorbing through exposure to green light. This green light promotes a phytochrome equilibrium that favors the active P_{fr} form, and green light is enough to activate phytochrome responses like seed germination. [13]. White light provides the full color spectrum that plants need for photosynthesis. This makes it an ideal light source for promoting germination and plant growth [8]. When cactus seeds are exposed to light, P_r is converted to P_{fr}. P_{fr} signaling induces the transcription of the gene encoding amylase. Which is an enzyme that breaks down starch stored in seeds into sugars, and then germination occurs [14]. Carotenoids and chlorophyll work together to receive light energy. Then, they are sent into the reaction center by the chlorophyll molecule, which serves to receive light energy and use it to create ATP and NADPH to use the power to make organic substances in the carbon dioxide fixation process [15]. It has been previously reported that light is a decisive factor in regulating the germination of many plant seeds. The study in *Mammillaria* spp. by Benítez-Rodríguez *et al*. [16] reported that red and white light had the highest germination percentage in contrast to *Epiphyllum phyllanthus* germinated under green light and darkness. Because phytochrome A responds to very low fluence and phytochrome B is in active form (P_{fr}) [17]. Red and white light gave *Cereus jamacaru* the highest germination percentage, and the height increased under red light. In comparison, blue light promotes chlorophyll and carotenoid content [18]. Red light exposure for 5 minutes per day for four days is sufficient for germinating *Cereus repandus* seeds [19]. Moreover, it was found that the seeds of *Nepenthes mirabilis* germinated first under white and red lights, and the seeds under green light will germinate into the final seeds. It was also recorded as the tallest seedling under red light [20].

Propagating cacti from seeds is a popular method because there are many seedlings of similar sizes and the opportunity to get plants with strange characteristics [21]. Some cactus seeds are dormant and grow slowly [7]. Light is an essential factor in seed germination. This is due to phytochromes, a group of photoreceptors that facilitate light germination and increase the number of bioactive gibberellins in seeds [22]. Seeds from columnar cacti were found to have a neutral photoblastic reaction. Globose and barrel-shape have positive photoblastic responses [23]. In addition, all plants need light for photosynthesis to produce carbohydrates (energy) for plant growth and development. The cactus should receive 10 - 14 hours of light [24]. The study of Takanori *et al*. [25] found that the growth rate and the number of shoots of *Nopalea cochenillifera* had the highest new growth under red light but the lowest under blue light. While growing, *Opuntia ficus-indica* was found that red and green light stimulated the development of cactus seedlings or LED light made cactus development [26]. Therefore, it can be assumed that red light stimulates seed germination. But blue light inhibits germination. In contrast, the red light and green light encourage cactus seedling development. This research aims to find suitable light colors to break dormancy and increase the growth of cactus seedlings in certain seasons with a short photoperiod and lower light intensity than natural light.

2. Materials and Methods

2.1 Seed collection

Seeds of three cactus species, *Astrophytum asterias*, *Echinocactus grusonii*, and *Turbinicarpus alonsoi*, were selected from the Chiang Mai, Thailand, cactus nursery. *Myrtillocactus geometrizans* was purchased from Mexico. The four species were collected from May to June 2023.

2.2 Imbibition test

The 100 dry seeds of each species were weighed in three replicates. Add 50 ml of distilled water and weigh the seeds until they reach a constant weight, which is considered the highest absorption. After that, the obtained data were calculated as percentage absorption (IP) according to the Lima and Meado formula [27].
IP = (seed imbibition weight – seed dry weight) × 100

2.3 Tetrazolium test for seed viability

One hundred seeds of each species/replication for three replicates were used to extract the embryos and soaked the seeds in water for 24 hours, after which the embryos were removed. The embryos were soaked in 0.6% tetrazolium solution for 16 hours at 30 ℃ [28]. Stained embryos were distinguished with a stereo microscope and according to the percentage of stained area. They must have more than 50% coloration to be considered viable seeds.

2.4 Seed germination experiment

Using seeds of 4 species of cactus, namely *E. grusonii*, *T. alonsoi*, *A. asterias*, and *M. geometrizans*, planted in 2-inch pots using peat moss: pumice in a ratio of 1:1 by volume. Add planting material in a volume of 150 ml. Slow-release fertilizer and Dinotefuran should be added to the planting material. Sow the seeds onto the planting material. Moreover, water mixed with fungicide (Captan) should be poured into a plastic box in a 75 ml volume. Close the lid of the container. Place it under LED light (red, blue, green light, use EVE lighting model A60 High power LED 6W 220V; White light uses LAMPTAN model Bulb bright 6W 6500K 220-240V) with 12 hours of light per day by planning the experiment in a completely randomized design, five treatments, four replications, 50 seeds per repetition, as follows: 1) natural light (control) (1490 $\mu mol/m^2/s$) 2) red light (109 $\mu mol/m^2/s$; 630 nm) 3) blue light (135 $\mu mol/m^2/s$; 470 nm) 4) green light (109 $\mu mol/m^2/s$; 520 nm) and 5) white light (1118 $\mu mol/m^2/s$; 447, 570 nm). As for the control method, seeds are planted in 2-inch pots inside a Daiso planting tray set with a lid and given water mixed with Captan. After that, place it under natural light from August to September.

2.5 α -Amylase assay

After sowing 100 cactus seeds, the samples were collected on day 1st and day 7th after sowing because the seeds were germinating. There was high α-amylase activity during this period. Then, the cactus seedlings were dried in a hot air oven at a temperature of 60℃. Then, grind 100 seeds thoroughly in the mortar and store them in a desiccant. The α-amylase activity was determined according to a method adapted to the Bernfeld method [29]. Weigh the cactus's powder at 0.05 g d.w. into centrifuges tube (15 ml). Then, take 8 ml of Tris buffer and shake with a shaker. After that, it was filtered with No. 1 filter paper, and the volume was adjusted to 8 ml using Tris buffer. Put the solution in storage. They were stored at -20 °C to analyze the amount of amylase enzyme. Measurement of reducing sugars obtained by hydrolyzing soluble starch with 3,5 dinitrosalicylic acid - reagent (DNS reagent) gives an orange color. The absorbance was measured using a spectrophotometer at a wavelength of 540 nm.

2.6 Chlorophyll A and Chlorophyll B content

The chlorophyll a and b content were estimated in μg/ml according to the method of Ghoochani *et al.* [30]. Cactus seedlings were washed and cleaned. Fresh seedlings were weighed to 0.2 g and then ground with a grinder. After that, the mixture was extracted with 80% acetone volume in 5 ml. If there was sediment, it was placed in a centrifuge at 2500 rpm for 10 minutes. Then, the absorbance value was measured using a spectrophotometer at wavelengths 663 and 647 nm. Chlorophyll a and chlorophyll b were calculated as follows: chlorophyll a (μg/ml) = $12.25_{A633} - 2.79_{A647}$ and Chlorophyll b (μg/ml) = $21.50_{A647} - 5.10_{A663}$

2.7 Plant growth analysis and statistical analysis

All growth parameters of the four species of cacti were determined 30 days after sowing by measuring the plants' exact size (the plants are not small and too large). There were as follows: Plant growth, i.e., stem diameter, stem length, and root length. Germination percentage (GP) was calculated from the formula of Kader [31] as follows: GP = (Number of seedlings that germination/Number of seeds sown) ×100. The seeding vigor index (SVI) was calculated by adapting the formula of Al-Ansari and Ksiksi [32], which was calculated from SVI = GP × (root length + stem diameter). Mean germination time (MGT) was calculated from the formula of Ellis and Roberts [33] as follows: MGT = $\sum(n \times d) / N$, where n = the number of seeds germinated on each day, d = number of days from the beginning of the test, and N = total number of seeds germinated at the termination of the experiment and color values by measuring through the Colorimeter application. The experiment followed the completely randomized designs (CRD) and was replicated fourth. ANOVA was conducted using the Statistix 10 program, and the mean was compared using the Least Significant Difference Test (LSD) at 95%.

3. Results and Discussion

3.1 Results

3.1.1 Quality of cactus seeds

The seed mass of 4 cacti species used for testing was 1.63 mg in *A. asterias*, 0.95 mg in *E. grusonii*, 0.56 mg in *M. geometrizans*, and 0.14 mg in *T. alonsoi*. The seeds of all four cacti species can absorb water (%imbibition) and were assessed for seed viability through TZ-testing (%) in various values as follows: in *A. asterias* had 0.456% imbibition and 63.33% embryo viability. In *E. grusonii*, there were 0.017% and 47.06%, respectively. In *M. geometrizans*, the imbibition percentage was 0.025%, and seed viability was 53.49%. *T. alonsoi* had 0.015% of imbibition and TZ-stained embryo viability, approximately 84.00% (Table 1).

Table 1. Quality of seed for all four cacti species.

Species	Seed mass (mg/100 seeds)	IP (%)	Seed viability (%)	Viability
Astrophytum asterias	1.63	0.456	63.33	
Echinocactus grusonii	0.95	0.017	47.06	
Myrtillocactus geometrizans	0.56	0.025	53.49	
Turbinicarpus alonsoi	0.14	0.015	84.00	

IP (%) = (seed imbibition weight – seed dry weight) × 100

3.1.2 germination of cactus seeds

The α-amylase activity in *A. asterias* seeds one day after sowing (DAS) showed that all LED lights (green, red, blue, and white) had higher α-amylase activities than natural light. At the 7^{th} DAS, α-amylase activity was the highest under the green light and increased to 0.22 μmole/ml, which was not different from natural light. In contrast, under the other light reduced (Figure 1A). The seed germination percentages under natural light were the highest at the 7^{th} DAS (89.00%). However, at the 28^{th} DAS, white light was the highest at 94.50%, which was not different from natural light (Figure 1B).

At the first DAS, the α-amylase activity in *E. grusonii* seeds showed that blue light had the highest averaging 0.32 μmole/ml (Figure 1C). The 7^{th} DAS, α-amylase activity under natural, green, and white light was higher than that under other colors. Regarding the germination percentage, at the 7^{th} DAS, blue light was the highest at 55.00%. After that, at the 28th DAS, red light was the highest at 94.50% but not different from blue light at 94.00% (Figure 1D).

The α-amylase activity in *M. geometrizans* under red light had the highest 0.16 µmole/ml (Table 1E). After the 7th DAS, α-amylase activity was the highest under natural light, increasing to 0.17 µmole/ml. Nevertheless, it was not significantly different from the red, blue, and green LED lights. The germination percentage at the 7th DAS with white light was the highest, at 34.50%. On the 28th DAS, the red light was the highest at 74.00%, but it was not different from natural light (Figure 1F).

The α-amylase activity in *T. alonsoi* under red and natural light had the highest 0.13 and 0.12 µmole/ml, but it was insignificant from blue LED light (Figure 1G). At the 7th DAS under red light, it increased to 0.22 µmole/ml. Furthermore, it was not significant from the white LED light and natural light. The germination percentage at the 7th DAS in white light was the highest at 34.50%, but the 28th DAS in natural light was the highest at 59.00%. However, it was not different from red and white lights (58.50% and 53.00%, respectively) (Figure 1H).

Mean germination time (MGT) in *A. asterias* showed that red light was the fastest at 4.29 days, but blue light was the slowest at 5.32 days (Table 2). *E. grusonii* and *M. geometrizans* likely germinated faster than others under red, blue, and white LED lights. In contrast, natural light had the slowest germination in *E. grusonii*, *M. geometrizans*, and *T. alonsoi*.

3.1.3 growth of cacti seedlings

Plant growth of 4 cacti was the greatest when culture under natural and white LED light, as shown in Table 2 and Figure 2. *A. asterias* showed that under natural light, it had the highest stem diameter (4.80 mm), root length (5.50 mm), seedling vigor (936.20), chlorophyll a and b content (4.76 µg/ml and 4.00 µg/ml, respectively). In contrast, it had the least plant height, 7.25 mm (Table 2).

In *E. grusonii*, under natural light and white light, there were the greatest stem diameters (3.53 and 3.33 mm); however, natural light had the lowest height (9.38 mm). While white light increased root length (8.00 mm) and seedling vigor (1025.00) (Table 2). As for chlorophyll, a and b content were not statistically different in the range of 2.17 – 2.75 µg/ml and 2.08 – 2.50 µg/ml, respectively.

In *M. geometrizans* under natural light had the maximum stem diameter (2.94 mm), root length (2.88 mm), seedling vigor (632.72), chlorophyll a and b content (1.13 µg/ml and 1.27 µg/ml, respectively). Nevertheless, it caused the plant's lowest height to be 6.25 mm (Table 2).

T. alonsoi, under natural light, had the most oversized stem diameter (1.85 mm), seedling vigor (199.60), and chlorophyll a and b content (2.34 µg/ml and 3.48 µg/ml, respectively) but caused the least plant height (5.25 mm). The white light caused the maximum root length to be 2.00 mm (Table 2)

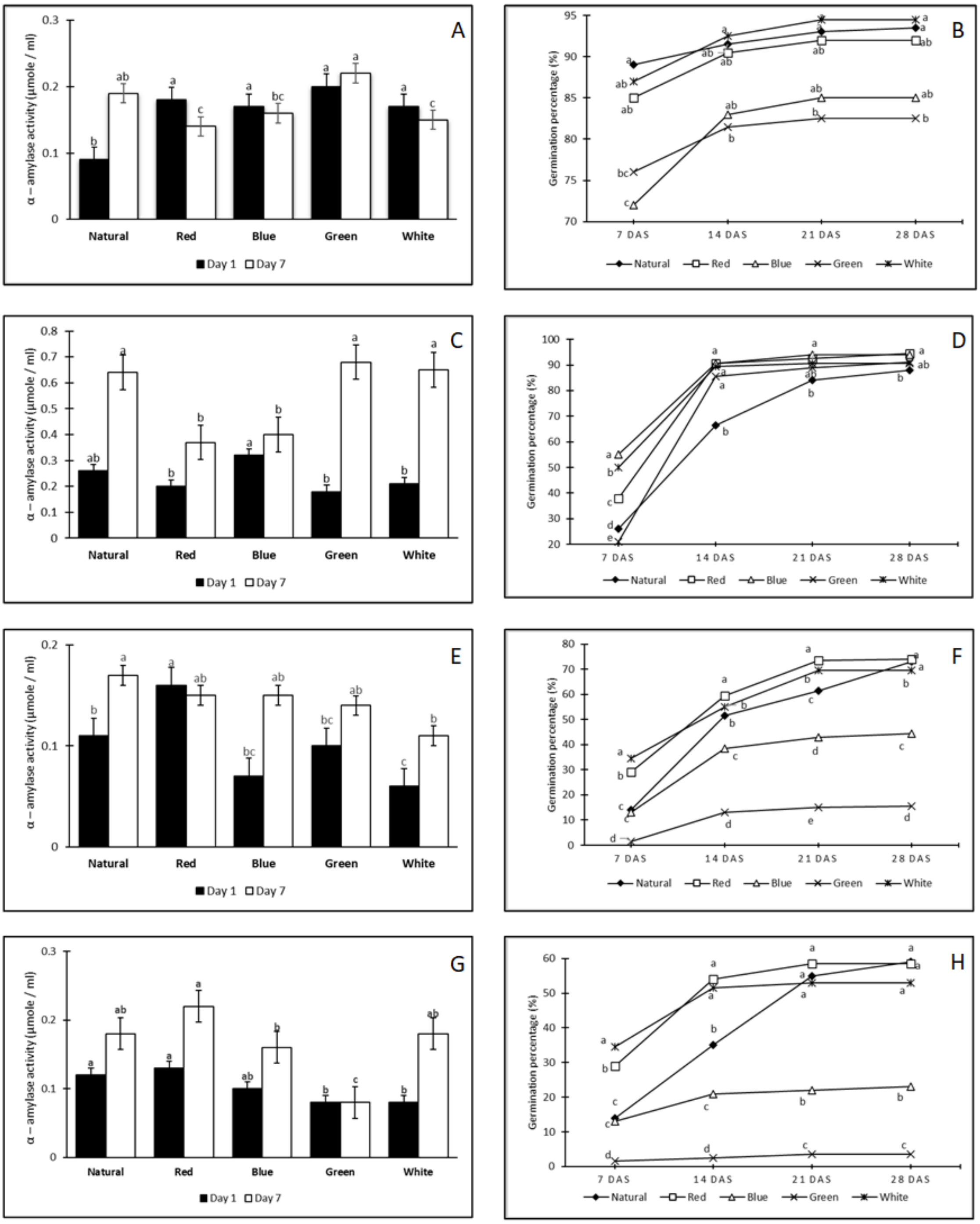

Figure 1. α-amylase activity on the 1st and the 7th DAS seeds in *A. asterias* (A), *E. grusonii* (C), *M. geometrizans* (E), and *T. alonsoi* (G); and the germination percentage of cactus in *A. asterias* (B), *E. grusonii* (D), *M. geometrizans* (F) and *T. alonsoi* (H).

Table 2. Cactus seedlings grow after giving different light colors for one month.

Treatment	MGT (days)	Stem diameter (mm)	Plant height (mm)	Root length (mm)	SVI	Chl. a (µg/ml)	Chl. b (µg/ml)
Astrophytum asterias							
Nature	4.70^{ab}	4.80^{a}	7.25^{d}	5.50^{a}	963.20^{a}	4.76^{a}	4.00^{a}
Red	4.29^{b}	2.93^{c}	15.13^{a}	2.75^{c}	463.48^{d}	2.17^{c}	2.08^{c}
Blue	5.32^{a}	3.03^{c}	12.13^{b}	3.50^{b}	544.70^{c}	2.50^{bc}	2.36^{bc}
Green	4.83^{ab}	2.54^{d}	14.13^{a}	2.50^{c}	415.23^{d}	1.52^{d}	1.63^{d}
White	4.61^{ab}	4.10^{b}	10.75^{c}	3.75^{b}	742.00^{b}	2.80^{b}	2.59^{b}
%CV	12.67	8.23	10.99	18.18	10.00	10.08	8.32
LSD$_{0.05}$	0.91	0.29	1.32	0.66	63.52	0.50	0.38
Echinocactus grusonii							
Nature	10.89^{a}	3.53^{a}	9.38^{c}	6.00^{b}	838.3^{b}	2.75	2.50
Red	7.15^{c}	2.06^{c}	20.88^{a}	4.38^{cd}	582.48^{c}	2.22	2.08
Blue	7.49^{c}	2.54^{b}	15.25^{b}	5.50^{bc}	742.07^{b}	2.36	2.28
Green	9.44^{b}	1.66^{d}	20.13^{a}	3.38^{d}	446.48^{d}	2.17	2.17
White	7.46^{c}	3.33^{a}	13.63^{b}	8.00^{a}	1025.00^{a}	2.44	2.43
%CV	6.27	8.07	10.61	20.51	14.90	15.80	14.13
LSD$_{0.05}$	0.80	0.21	1.70	1.13	109.99	ns	ns
Myrtillocactus geometrizans							
Nature	12.51^{a}	2.94^{a}	6.25^{d}	2.88^{a}	632.72^{a}	1.13^{a}	1.27^{a}
Red	8.64^{b}	1.60^{d}	11.63^{a}	2.50^{ab}	316.57^{c}	0.92^{ab}	1.12^{ab}
Blue	9.77^{b}	1.90^{c}	9.50^{b}	2.25^{bc}	366.60^{c}	0.79^{b}	0.97^{b}
Green	10.13^{ab}	1.49^{d}	9.75^{b}	2.00^{c}	238.42^{d}	0.93^{ab}	1.12^{ab}
White	9.52^{b}	2.71^{b}	8.13^{c}	2.13^{bc}	505.89^{b}	0.91^{ab}	1.12^{ab}
%CV	17.87	8.69	7.87	16.48	5.27	18.83	13.78
LSD$_{0.05}$	2.72	0.18	0.72	0.39	55.87	0.32	0.28
Turbinicarpus alonsoi							
Nature	12.39^{a}	1.85^{a}	5.25^{d}	1.75^{b}	199.60^{a}	2.34^{a}	3.48^{a}
Red	8.09^{b}	1.14^{d}	11.13^{a}	1.50^{c}	108.50^{b}	2.04^{b}	3.24^{b}
Blue	8.33^{b}	1.26^{c}	8.75^{b}	1.63^{bc}	60.60^{c}	2.03^{b}	3.23^{b}
Green	7.88^{b}	0.54^{e}	9.00^{b}	1.13^{d}	11.38^{d}	nd	nd
White	7.11^{b}	1.63^{b}	7.25^{c}	2.00^{a}	179.55^{a}	2.01^{b}	3.18^{b}
%CV	21.07	4.40	12.15	11.81	14.66	2.76	2.39
LSD$_{0.05}$	2.78	0.06	1.02	0.19	24.73	0.11	0.15

$^{a, b, c, d, e}$ Letters in the same column differ significantly when they are different letters ($p < 0.05$).

ns were not statistically different, and nd were not detected.

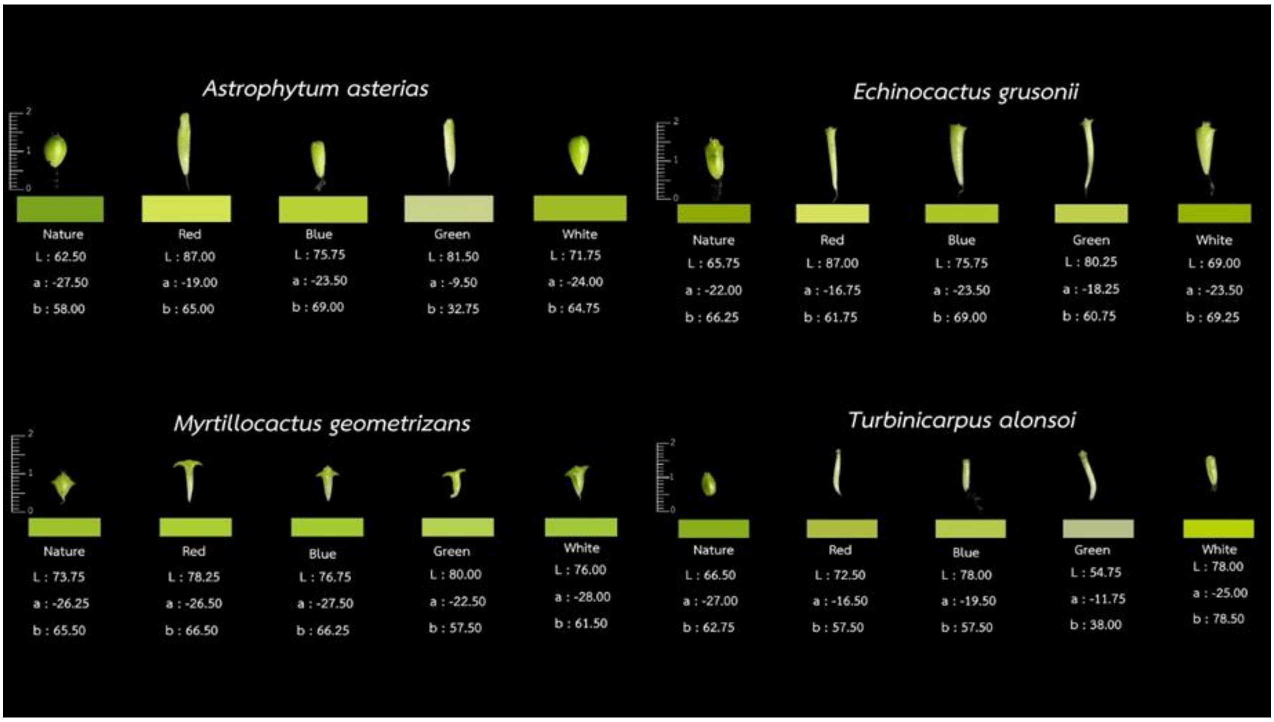

Figure 2. Morphological growth and color value of 4 cacti seedlings.
L* value means brightness value (large L* value shows high brightness, small L* value shows low brightness)
The value a* means the value indicating green and red (red value is (+), green value is (-)).
The value b* means the value indicating blue and yellow (the yellow value is (+), and the blue value is (-)).

3.2 Discussion

Seeds of cacti species can absorb water, and there was a high viability of more than 50% except for *E. grusonii*. The cactus seeds used were not hard because the hard seeds did not imbibe water after one or two days and remained hard to the touch. In contrast, non-hard seeds rapidly imbibed and became soft [34]. Additionally, cactus seeds may possess physiological dormancy because of high viability. There were seed dormancy as a germination percentage gradually increased in natural light. At the same time, various colors of LED light can stimulate germination. *A. asterias* exposed to white light had the highest germination percentage, but green light had the highest α-amylase activity. In *E. grusonii*, *M. geometrizans*, and *T. alonsoi*, germination percentage and α-amylase activity were the highest under red light. In addition, all four cacti species had the lowest mean germination time under red light. The red light stimulates P_r to be converted to P_{fr}, causing a gene encoding that stimulates the synthesis of α-amylase, an enzyme that breaks down starch stored in seeds. It is a sugar transferred to the embryo, where it germinates [14]. This is consistent with the study of Lal and Sachan [36], which declared that red light showed the highest germination percentage *Nepenthes mirabilis* seeds germinated fastest under red light [20]. White light provides the entire color spectrum, making it an ideal source for promoting germination [8]. Consistent with the study of Benítez-Rodríguez *et al*. [16], red light and white light resulted in *Mammillaria* sp. having the highest germination percentage. The green light promotes phytochrome balance, which supports the active P_{fr} form and is sufficient to stimulate the phytochrome response used in seed germination [13]. This is consistent with Simão *et al*. declaring that in *Epiphyllum phyllanthus* germinated under green light and darkness [17]. In terms of growth, it was found that *A. asterias* and *M. geometrizans* had the highest seedling vigor under natural light. It also had the highest stem diameter, plant height, and root length. They had the highest chlorophyll a and chlorophyll b. In *E. grusonii* and *T. alonsoi* under natural light, with the highest stem diameter and plant height. In addition, it increases chlorophyll a and chlorophyll b. However, white light has the maximum root length and seedling vigor because natural light comprises wavelengths. Furthermore, all frequencies of the visible light spectrum, including natural light, had a light intensity closest to (3040 lux) to the light intensity required by cactus seedlings, which is 5000 lux [37]. White light provides the full color spectrum that plants need for photosynthesis. This makes it an ideal plant-growth light source [11]. This is consistent with research the study of Dangudom *et al*. [38], who found that natural light with 60% blackout, two layers of silver and white light made *Gymnocalycium mihanovichii* and *A. asterias* had high yields and beautiful stem quality. Thus, white light can be used instead of natural light in the short photoperiod season. Therefore, it can be concluded that red, green, and white light can stimulate germination and release seed dormancy. In addition, white light helps cacti grow, as does natural light.

4. Conclusions

It can be concluded that red, green, and white light can stimulate germination and release seed dormancy. In addition, white light helps cacti grow, as does natural light.

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