



Effect of Plant Growth Regulators on *In Vitro* Shoot and Root Induction and Acclimatization of *Gymnocalycium mihanovichii* LB2178 Agua Dulce (Cactaceae)

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Abstract: *Gymnocalycium mihanovichii* LB2178 Agua Dulce is a popular cactus still in demand, known for its distinctive shape and ease of growth. It can be propagated by tissue culture to produce strong, disease-free plants. Therefore, the aim of this research was 1) to study the effect of BA and IBA hormones and types of explants on the growth rate of *G. mihanovichii* LB2178 Agua Dulce cactus. and 2) to study suitable planting materials for survival and growth of *G. mihanovichii* LB2178 Agua Dulce cactus under natural conditions. After 3 months of culture, the results showed that the explant sectioned longitudinally were cultured on MS medium supplemented with 0 and 2 mg/L BA gave the highest survival, 100%, and shoot induction, 86.67%, the explant sectioned transversally were cultured on MS medium supplemented with 1 mg/L BA gave the highest number of shoot, 4.20 shoots/explant, and shoot length, 0.36 mm respectively, significant difference at $p \leq 0.05$. Following three months of cultivation, the findings indicated that shoot explants cultured on MS medium supplemented with 1 mg/L IBA showed the highest root induction (91.67%), average number of roots (20.47 roots/explant), and average root length (7.63 mm), with significant differences at $p \leq 0.05$. After 3 months, the rooted plants were removed from the tissue culture room and transferred to sterile plastic pots. The result showed that *G. mihanovichii* LB2178 Agua Dulce grown in potting soil: sand, showed the highest survival rate of 100%.

Keywords: *Gymnocalycium mihanovichii* LB2178 Agua Dulce; benzyladenine; indole-3-butyric acid; acclimatization; *in vitro*

1. Introduction

Cacti are one of the plant groups with high morphological diversity, with 127 genera and approximately 1,430-2,000 species reported worldwide [1]. Most of them are densely distributed in South America, particularly Mexico, and in North America, including the United States and Canada [2]. Most cacti are cultivated as ornamental plants with high market value [3]. In addition, some cacti are used as food plants, eaten as fruits, and used in the food industry for livestock [4]. They are used as ingredients in food menus or processed foods, and local people in Mexico like to raise hummingbirds (*Mellisuga helenae*) to help pollinate cacti and increase fruiting rates, helping secure the local people's

food source. Cacti are slow-growing plants that can thrive in a variety of environments, especially in desert areas, making them among the most beautiful desert plants in the world.

Cactus has special characteristics. It has its own unique characteristics: a succulent stem that is round, cylindrical, or serrated, growing as a single stem or a clumping stem; and leaves that have evolved into thorns to reduce water loss. It also has a wide variety of thorn types, including needle-like, ribbed, paper, and hairy thorns. In addition, cacti are plants with beautiful flowers in various shapes, such as funnel-, bell-, dish-, and tubular-shaped flowers, as well as stems and flowers in various colors, such as yellow, purple, pink, red, white, and cream [5]. Because of these unique qualities, cacti are now among the most widely used ornamental plants in homes and offices. As a result, 130 species of cacti are imported and exported to 182 countries, with an average annual market value of 11–60 billion US dollars. The value is continuously increasing every year [6, 7].

Cacti are among the most popular ornamental plants grown and cultivated in Thailand. *Gymnocalycium mihanovichii* is one of the *Gymnocalycium* genus's cactus family, which is ranked first in popularity (average 55%) among cactus lovers compared to other cactus species (45%). It has a total value of up to 2 million baht [3]. This is because the plants have unique stem characteristics, stem color, thorn characteristics, and highly diverse flower shape and color. Consumers, especially those interested in new plant varieties with specific qualities that differ from the original varieties, frequently influence market trends in ornamental plants and the cactus family. As a result, the market value of the new plant varieties increases several times. Furthermore, since *G. mihanovichii* LB2178 Agua Dulce is a lovely decorative plant that may be multiplied by dividing the shoots or, if a large quantity is required, by seed, it is also much sought after in the market. However, the propagation process is slow and susceptible to plant diseases [8]. Currently, researchers are using plant tissue culture techniques to propagate plantlets and increase the number of plants in a short period of time. According to the report by Rittirong *et al.* [9], 0.5 cm shoots of *Gymnocalycium* spp. were cultured on MS medium with different concentrations of NAA (0.0, 1.0, 2.0, 3.0, and 4.0 mg/L) and BA (0.0, 1.0, 2.0, 3.0, and 4.0 mg/L). After 3 months of culture, the efficiency of the tissue culture approach for producing a large number of *G. mihanovichii* LB2178 Agua Dulce. The application of cytokinin can awaken previously dormant axillary buds from the constraints of apical dominance [10]. Regarding shoot rooting, auxins are the most commonly used exogenous phytohormones for root development, and IBA is more effective than NAA alone or in combination with IBA [11].

We cannot find any reports on *in vitro* shoot and root induction in *G. mihanovichii* LB2178 Agua Dulce, or on the acclimatization and hardening of rooted plants. In commercial plantations of this species, *ex vitro* propagation using stem cuttings takes approximately 1 month to induce root growth, although the exact duration varies across cultivation stages. In this research, the effect of BA and IBA hormones and types of explants on the increase of *G. mihanovichii* LB2178 Agua Dulce cactus was studied, as well as the planting materials that are suitable for the survival and growth of *G. mihanovichii* LB2178 Agua Dulce cactus.

2. Materials and Methods

2.1 Plant materials

The 1 cm seedlings of *G. mihanovichii* LB2178 Agua Dulce were cut from the shoot and divided into two types of parts: a longitudinal cut and a cross-sectional piece measuring 3–4 mm. In the tissue culture room at the science center in Nakhon Si Thammarat Rajabhat University.

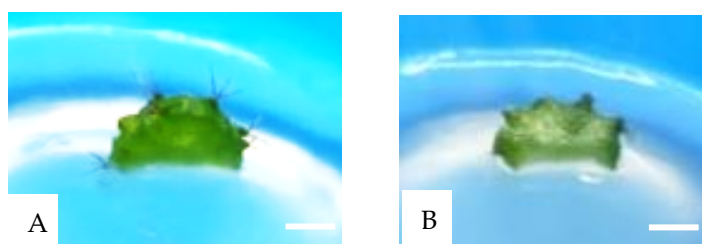


Figure 1. The 1 cm seedlings of *G. mihanovichii* LB2178 Agua Dulce were cut off the shoot and divided into two types of parts (bar = 1 cm) (A) a longitudinal cut (B) a cross-sectional cut

2.2 Effect of different explant fragmentation and different concentrations of Benzyladenine (BA) on shoot induction of *G. mihanovichii* LB2178 Agua Dulce

The 0.5 cm shoot explants were cultured on MS medium containing 3 BA concentrations (0, 1, and 2 mg/L). All culture media were supplemented with 30 g/L sucrose and 8 g/L agar. The media were adjusted to a pH of 5.7. For three months, the cultures were subcultured every 4 weeks on the same medium component, kept at 25±2 °C under 3000 lux light intensity for 14 hours per day. The survival rate, shoot induction percentage, number of shoots per explant, and shoot length were evaluated after 3 months of culture.

2.3 Effect of different concentrations of Indole-3-butyric acid (IBA) on root induction of *G. mihanovichii* LB2178 Agua Dulce

The shoot explants from experiment 2.2 were cultured on MS medium containing 5 IBA concentrations (0, 0.5, 1, 1.5, and 2 mg/L). All culture media were supplemented with 30 g/L sucrose and 8 g/L agar. The media were adjusted to a pH of 5.7. For three months, the cultures were subcultured every 4 weeks on the same medium component, kept at 25±2 °C under 3000 lux light intensity for 14 hours per day. Root induction, average number of roots, and average root length were evaluated after 3 months of culture.

2.4 Acclimatization and hardening of rooted plants

After 3 months, the rooted plants were removed from the tissue culture room and adjusted to room temperature for 2 days. Plantlets with well-developed roots were thoroughly washed in tap water and then transplanted into sterile plastic pots filled with a 1:1 mixture of three sterilized soil formulas: potting soil and pumice, potting soil and sand, and potting soil, sand, and pumice. Put 25 grams of each soil formula in 15 pots. Plastic bags were placed over the pots. After adjusting it with a rubber band for seven days, move the cactus to the greenhouse to acclimate. Observe and record the percentage survival after 1 month in culture.

2.5 Statistical analysis

The experimental design and statistical analysis were conducted using a fully randomized design with three replicates, each containing 10 explants. Using Duncan's Multiple Range Test (DMRT), the mean differences were compared after ANOVA.

3. Results and Discussion

3.1 Effect of different explant fragmentation and different concentrations of Benzyladenine (BA) on shoot induction of *G. mihanovichii* LB2178 Agua Dulce

The 0.5 cm shoot explants were cultured on MS medium containing 3 BA concentrations (0, 1, and 2 mg/L). The result found that the explant sectioned longitudinally, cultured on MS medium containing concentrations of BA (0 and 2 mg/L), gave the highest survival, 100%, followed by the explant sectioned transversally, which was cultured on MS medium containing concentrations of BA (0 and 2 mg/L), and the explant sectioned longitudinally were cultured on MS medium containing concentrations of BA (1 mg/L). The explant sections transversally were cultured on MS medium containing concentrations of BA (1 mg/L), 86.67%, 80.00%, and 73.33%, respectively, with a significant difference at $p \leq 0.05$. Shoot induction, the result found that the explant sectioned longitudinally were cultured on MS medium containing concentrations of BA (2 mg/L) gave the highest shoot induction, 86.67%, followed by the explant sectioned transversally were cultured on MS medium containing concentrations of BA (1 and 2 mg/L) and the explant sectioned longitudinally were cultured on MS medium containing concentrations of BA (1 mg/L), 76.67% and 56.57% respectively, significant difference at $p \leq 0.05$. Number of shoot, the result found that the explant sectioned transversally were cultured on MS medium containing concentrations of BA (1 mg/L) gave the highest number of shoot, 4.20 shoots/explant, followed by the explant sectioned transversally were cultured on MS medium containing concentrations of BA (2 mg/L), the explant sectioned longitudinally were cultured on MS medium containing concentrations of BA (2 mg/L) and the explant sectioned longitudinally were cultured on MS medium containing concentrations of BA (1 mg/L), 2.20 shoots/explant, 2.13 shoots/explant and 1.33 shoots/explant respectively, significant difference at $p \leq 0.05$. Shoot length, the result found that the explant sectioned transversally, cultured on MS medium containing concentrations of BA (1 mg/L), gave the highest shoot length, 0.36 cm, followed by the explant sectioned longitudinally were cultured on MS medium containing

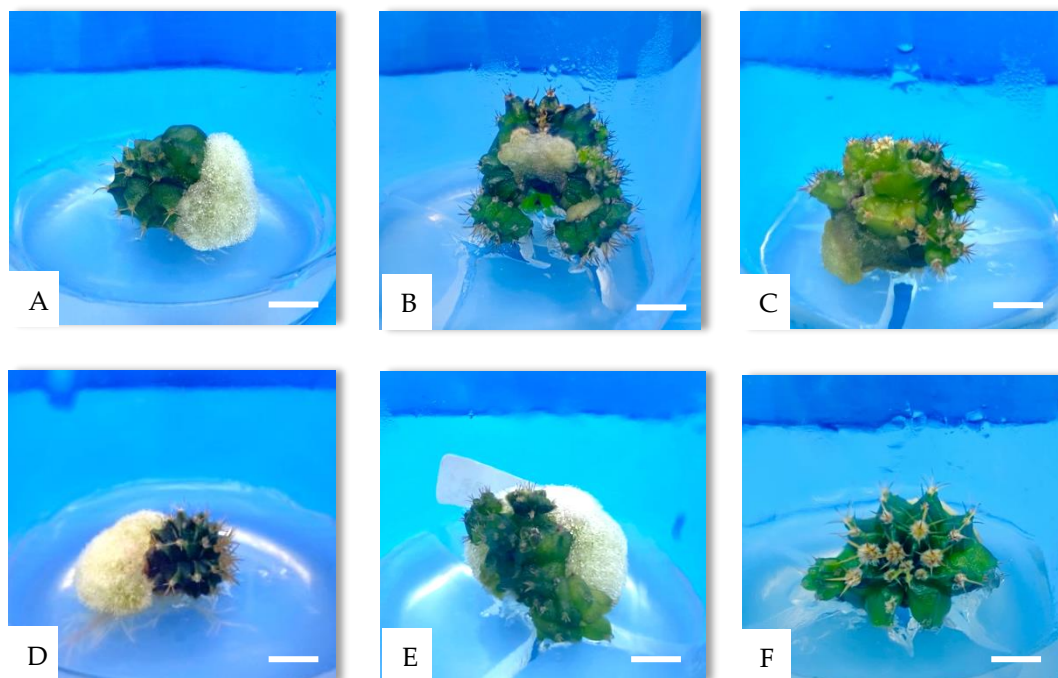
concentrations of BA (2 mg/L), the explant sectioned transversally, which was cultured on MS medium containing concentrations of BA (2 mg/L). The explant sections longitudinally, which were cultured on MS medium containing concentrations of BA (1 mg/L), 0.19 cm, 0.17 cm, and 0.14 cm, respectively, showed a significant difference at $p \leq 0.05$. (Table 1 and Figure 2)

The study found that the induction of shoots of *G. mihanovichii* LB2178 Agua Dulce is affected by high BA concentrations and explant fragmentation. Because it has been observed that changing explant polarity can lead to faster growth responses, the explant orientation data presented here contradict these findings. A greater area of contact with the culture medium, its nutrients, and the PGRs it contains could be one factor contributing to explants in vertical posture having higher %E values [12]. According to Bonga [13], the findings of this study are consistent with the observation that very small explants frequently do not grow at all, thereby lowering statistical multiplication rates. Other techniques, such as fragmentation and different explant orientations in the culture media, can also be considered to boost *in vitro* shoot output and reduce the number of original plants used. When PGRs such as auxins and cytokinins are used, the number of shoots often increases. [14] According to Silva [15], the longitudinal cuts of *Melocactus glaucescens* cultured on MS medium supplemented with different concentrations of BA and NAA showed higher growth and shoot induction than the transverse cuts. When BA or kinetin (KIN) was combined with naphthalene acetic acid (NAA), BAP produced more shoots than KIN, according to an analysis of the morphogenic responses of *M. glaucescens* explants to PGRs. Nevertheless, branches with BAP concentrations of 4.44, 8.88, and 17.76 μM differed morphologically from the control plants. According to Masakee *et al.* [16] *Haworthia* callus cultured on MS medium supplemented with 1.0 mg/L BA showed 75.00% green spot formation and 48.75 green spots. Similarly, Marhri *et al.* [17] reported that the concentration of BAP growth hormone had a direct effect on shoot proliferation, as indicated by analysis of variance, which showed significant differences in the means of the four groups. When compared to the other doses studied, BAP alone produced the most efficient number of regenerated shoots from a single wild prickly pear shoot, averaging 19.42 shoots at a concentration of 5 mg/L. Furthermore, BA hormone is crucial for plant regeneration and shoot induction, as well as for cell division and growth, lateral bud formation and development, seed germination, and protein synthesis. The effects of hormone concentration revealed that, whereas intermediate doses often permitted increased shoot formation, higher concentrations were less effective. It is well known that reactions to exogenous hormones vary depending on the species and explant source. In purple pitahaya (*Hylocereus costaricensis*), higher hormone concentrations have been shown to affect calluses while blocking shoot development. [18]. The *Turbinicarpus mombergeri* shoots were cut longitudinally, and the segments were transferred to media containing 2.22 or 4.44 μM benzyladenine to induce shooting. The generated shoots were highly hydrated. [19] According to Huong [20], all explants in the medium culture with BA added showed improved shoot formation after 6 weeks of culture, with 100% shoot induction. As the concentration of BA grew from 0.5 to 1.0 mg/L, the number of shoots per explant and shoot height increased. However, as the concentration of BA climbed to higher concentrations (1.5 and 2.0 mg/L), it gradually declined. The trial with MS media supplemented with 1.0 mg/L BA yielded the highest number of shoots, shoot height, and shoot regeneration rate (43 shoots per explant, 9.4 mm, and 100%, respectively).

Table 1. Effect of different explant fragmentation and different concentrations of BA on the shoot induction of *G. mihanovichii* LB2178 Agua Dulce after 3 months of culture

Culture medium	Explant fragmentation	Survival (%)	Shoot induction (%)	Number of shoots (shoots/explant)	Shoot Length (cm)
MS	Sectioned transversally	86.67 ^{ab}	0.00 ^c	0.00 ^c	0.00 ^c
	Sectioned longitudinally	100.00 ^a	0.00 ^c	0.00 ^c	0.00 ^c
MS + BA 1 mg/L	Sectioned transversally	73.33 ^b	76.67 ^{ab}	4.20 ^a	0.36 ^a
	Sectioned longitudinally	80.00 ^{ab}	56.57 ^b	1.33 ^{bc}	0.14 ^b
MS + BA 2 mg/L	Sectioned transversally	86.67 ^{ab}	76.67 ^{ab}	2.20 ^b	0.17 ^b
	Sectioned longitudinally	100.00 ^a	86.67 ^a	2.13 ^b	0.19 ^b
	F-test	*	*	*	*
	C.V.(%)	36.87	60.57	14.87	11.06

* significant difference at $p \leq 0.05$

**Figure 2.** Characteristics of shoot formation of *G. mihanovichii* LB2178 Agua Dulce were cultured on MS medium containing 3 different concentrations of BA after culturing for 3 months (bar = 1 cm) (A) MS: Sectioned transversally (B) MS: Sectioned longitudinally (C) MS+1 mg/L BA: Sectioned transversally (D) MS+1 mg/L BA: Sectioned longitudinally (E) MS+2 mg/L BA: Sectioned transversally (F) MS+2 mg/L BA: Sectioned longitudinally

3.2 Effect of different concentrations of Indole-3-butyric acid (IBA) on root induction of *G. mihanovichii* LB2178 Agua Dulce

The shoot explants from experiment 2.2 were cultured on MS medium containing 5 IBA concentrations (0, 0.5, 1, 1.5, and 2 mg/L). The result found that the shoot explant were cultured on MS medium containing concentrations of IBA (1 mg/L) gave the highest root induction, 91.67%, followed by the shoot explant were cultured on MS medium containing concentrations of IBA (0.5 mg/L), the shoot explant were cultured on MS medium containing concentrations of IBA (1.5 mg/L), the shoot explant were cultured on MS medium containing concentrations of IBA (2 mg/L). The shoot explants were cultured on MS medium containing

concentrations of IBA (0 mg/L), 83.33%, 81.67%, 75.00%, and 70.00%, respectively, with a significant difference at $p \leq 0.05$. Average number of root, the result found that the shoot explant were cultured on MS medium containing concentrations IBA (1 mg/L) gave the highest average number of root, 20.47 roots/explant, followed by the shoot explant were cultured on MS medium containing concentrations of IBA (0.5 mg/L), the shoot explant were cultured on MS medium containing concentrations of IBA (1.5 mg/L), the shoot explant were cultured on MS medium containing concentrations of IBA (2 mg/L). The shoot explants were cultured on MS medium containing concentrations of IBA (0 mg/L), 14.73 roots/explant, 10.47 roots/explant, 7.87 roots/explant, and 4.87 roots/explant, respectively, with a significant difference at $p \leq 0.05$. Average length of root, the result found that the shoot explant were cultured on MS medium containing concentrations of IBA (1 mg/L) gave the highest average length of root, 7.63 mm, followed by the shoot explant were cultured on MS medium containing concentrations of IBA (0.5 mg/L), the shoot explant were cultured on MS medium containing concentrations of IBA (1.5 mg/L), the shoot explant were cultured on MS medium containing concentrations of IBA (2 mg/L) and the shoot explant were cultured on MS medium containing concentrations of IBA (0 mg/L), 6.57 mm, 5.38 mm, 4.62 mm and 4.03 mm respectively, significant difference at $p \leq 0.05$. (Table 2 and Figure 2)

The study found that IBA concentration affects root induction in *G. mihanovichii* LB2178 Agua Dulce. Since IBA is a member of the auxin family of plant hormones and a component of many commercial horticulture plant rooting products. In addition to hastening root production, it is applied to a variety of crops to promote fruit growth and flower development. In plant tissue culture, IBA and other auxins are used to initiate root formation *in vitro*. Regarding shoot rooting, auxins are the most commonly used exogenous phytohormones for root development, and IBA is more effective than NAA alone or in combination with IBA [11]. In the current investigation, kinetin was utilized solely to direct organogenesis toward root development. The optimal outcome was achieved with kinetin at 1.5 mg/L, yielding an average of 9.08 roots. The percentage of rooted explants reached 100% when harvested, and regenerated *Selenicereus megalanthus* shoots were added to rooting media supplemented with 0.5 mg/L IBA; this percentage decreased progressively with higher IBA concentrations (1.0–2.0 mg/L). The maximum percentage of rooted explants, number of roots per explant, and root length were seen on a half-strength MS medium supplemented with 0.5, 1.0, 1.5, and 2.0 mg/L IBA. Every culture was incubated at $25 \pm 2^\circ\text{C}$ with a 16-hour photoperiod ($10\text{--}15 \mu\text{Em}^{-2}\text{s}^{-1}$). After a week of culture, IBA 1.0 mg/L showed signs of rooting. 100% of the shoots had rooted two weeks following transplant [21]. The best rooting response for the *Haworthia retusa* shoot was observed in half-strength MS medium supplemented with 0.5 mg/L IBA. This medium formulation produced the largest mean root number (5 roots/explant) and the highest rooting rate (100%) [22]. Auxins such as IBA can be added to the culture medium to aid root development in succulent plants. It is important to strike a balance between the auxin concentration and the succulent species' requirements to achieve successful rooting [23]. Furthermore, even in the absence of growth hormones, the organ developed sufficiently after three weeks, suggesting that this species does not require root development stimulation. In actuality, plants have a natural ability to regenerate missing sections after damage, and the agar media further promotes the growth of adventitious roots [24, 18].

Table 2. Effect of different concentrations of IBA on root induction of *G. mihanovichii* LB2178 Agua Dulce after 3 months of culture

Culture medium	Root induction (%)	Average number of roots (roots/explant)	Average length of root (cm)
MS	70.00 ^b	4.87 ^e	4.03 ^d
MS + IBA 0.5 mg/L	83.33 ^{ab}	14.73 ^b	6.57 ^b
MS + IBA 1 mg/L	91.67 ^a	20.47 ^a	7.63 ^a
MS + IBA 1.5 mg/L	81.67 ^{ab}	10.47 ^c	5.38 ^c
MS + IBA 2 mg/L	75.00 ^{ab}	7.87 ^d	4.62 ^{cd}
F-test	*	*	*
C.V.(%)	29.00	30.00	23.00

*significant difference at $p \leq 0.05$

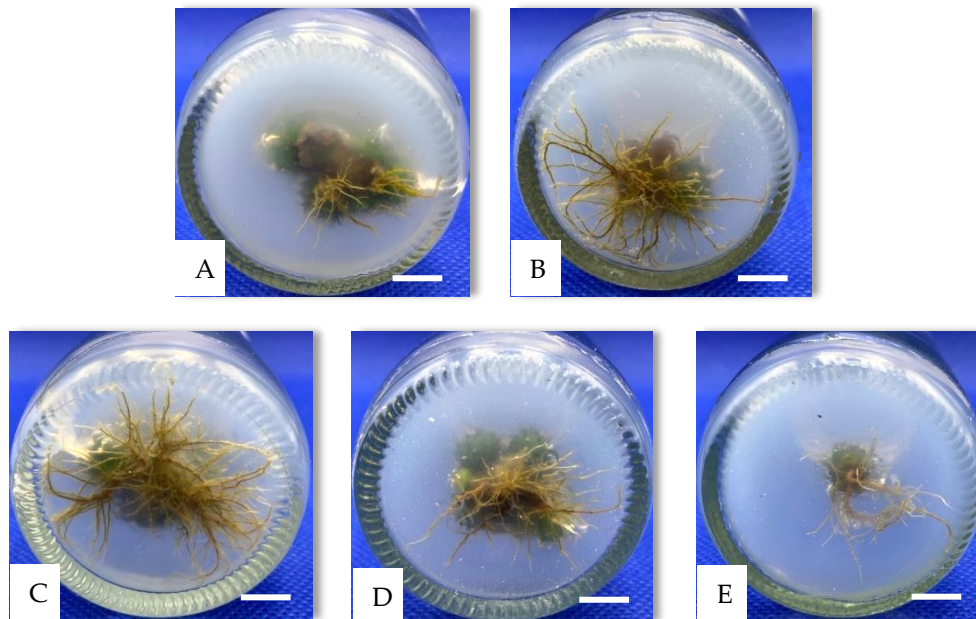


Figure 3. Characteristics of root formation of *G. mihanovichii* LB2178 Agua Dulce were cultured on MS edium supplemented with different concentrations of IBA after culturing for 3 months (bar = 1 cm) (A) MS (B) MS+0.5 mg/L IBA (C) MS+1 mg/L IBA (D) MS+1.5 mg/L IBA (E) MS+2 mg/L IBA

3.3 Acclimatization and hardening of rooted plants

After 3 months, the rooted plants were removed from the tissue culture room and placed in sterile plastic pots filled with a 1:1 mixture of three sterilized soil formulas: potting soil:pumice, potting soil: sand, or potting soil:sand:pumice. These pots were left for seven days before being moved to the greenhouse for a month of acclimatization. According to the findings, *G. mihanovichii* LB2178 Agua Dulce grown in potting soil: sand showed the highest survival rate of 100%, followed by potting soil:sand:pumice with 80% survival rate and potting soil: pumice with 75% survival rate. It was also found that a potting soil:sand ratio of 1:1 gave the highest average height of *G. mihanovichii* LB2178 Agua Dulce plants, 2.4 cm, after 2 months of growth (Figure 4). The study found that *G. mihanovichii* LB2178 Agua Dulce grown in potting soil (sand) showed the highest survival rate of 100%. This success can be ascribed to the Opuntiaceae's well-developed root structure, which permits a high capacity for rehydration. Desiccation can also be prevented by employing a progressive acclimatization technique, which involves applying a plastic cover for 2-3 weeks to raise external humidity and address anomalies. [17] According to the healthy regenerated *Caralluma diffusa* (Wight) N.E.Br. plantlets were removed from the growth tubes and placed in tiny plastic cups filled with sand, fertile soil, and vermicompost (1:1:1) in a controlled environment. The plantlets were then moved to large pots and allowed to acclimate to outdoor conditions over 4 weeks. Compared with their parents, *in vitro*-grown plants did not exhibit any discernible differences in physical traits, and over 98% of the plantlets were effectively acclimated. [25] After 4 weeks, the roots of *Selenicereus megalanthus* were removed from the culture media, properly cleaned with autoclaved, double-distilled water, and placed in sterile plastic containers with a 1:1 ratio of sterilized soil to sand. After being wrapped in polythene bags for ten to twelve days, the cups were moved to the greenhouse to acclimate. After 2 months, established plants were transferred to containers. After 2 months, 95% of the transferred plants survived. [21] In addition, because the planting material is an important factor in raising all species of cactus, "*Gymnocalycium*" requires soil with sufficient nutrients and good drainage, and the soil must not be too compact, as this can prevent the plant's roots from expanding into the bottom of the pot. Consequently, airy planting material is required. Most planters use a pot filled with crushed volcanic rock/cooking charcoal, cactus soil mixed with small volcanic rock, crab claw soil, perlite, coarse sand, Osmocote fertilizer, and Stargle. You can adjust the proportions and soil formulas to suit your specific

cultivation needs. The materials can also be adjusted according to what is available in each area. However, regardless of the formula used, the soil must drain well so it does not hold too much water [26]. According to the Digital Agriculture Database System and Sarapancactus, cacti should be planted in sandy loam soil, use fertile planting materials, or use mixed soil with abundant nutrients, with easy water flow, not water-retentive, and good moisture retention, and receive sufficient sunlight for cactus growth. This may be due to the limitation of the study period of cactus growth, so no difference in the diameter of the cactus stems was seen. This is evident in the low percentage of cactus seed survival. If the study period is extended, the percentage of survival may increase, leading to differences in the diameter of the cactus stems.

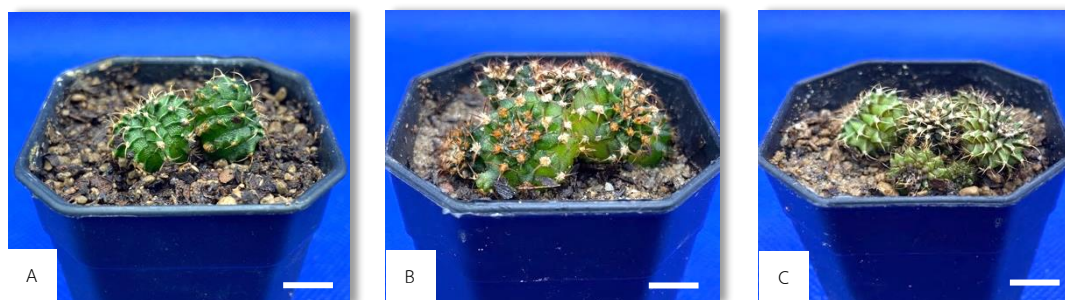


Figure 4. After culturing for a month, the seedlings of *G. mihanovichii* LB2178 Agua Dulce emerged in various growing media. (A) Soil: pumice ratio 1:1 (B) Soil: sand ratio 1:1 (C) Soil:sand:pumice ratio 1:1:1 (bar = 1 cm)

4. Conclusions

In vitro methods can be used to propagate *G. mihanovichii* LB2178 Agua Dulce. The longitudinally sectioned explants cultivated on MS media containing doses of BA (0 and 2 mg/L) exhibited the best survival rate (100%) and shoot induction rate (86.67%) after 3 months of culture, according to the data. The transversally sectioned explants grown on MS medium containing concentrations of BA (1 mg/L) exhibited the greatest shoot length (0.36 cm) and shoot number (4.20 shoots/explant). The shoot explants cultivated on MS medium containing concentrations of IBA (1 mg/L) produced the maximum root induction (91.67%), average number of roots (20.47 roots/explant), and average root length (7.63 cm) after 3 months of culture. The rooted plants were taken out and placed in sterile plastic pots for acclimation and hardening. The maximum survival rate of 100% was demonstrated by *G. mihanovichii* LB2178 Agua Dulce cultivated in a potting soil: sand mixture. Additionally, these methods can help meet commercial demands, thereby lessening the strain on *G. mihanovichii* LB2178 Agua Dulce natural populations. Other possibilities for the quick growth of *G. mihanovichii* LB2178 Agua Dulce include BA and IBA hormones.

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Author Contributions: A short paragraph specifying their individual contributions must be provided for research articles with several authors. The following statements should be used “Conceptualization, P.R. and S.T.; methodology, P.R.; software, S.T.; validation, P.R., S.T. and T.K.; formal analysis, P.R.; investigation, P.R.; resources, P.R. and T.K.; data curation, P.R.; writing—original draft preparation, P.R.; writing—review and editing, S.T.; visualization, T.K.; supervision, S.T.; project administration, P.R. and S.T. All authors have read and agreed to the published version of the manuscript.” Please turn to the CRediT taxonomy for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported..

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