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Research

Effect of Gibberellic Acid and Eggshell on *Hylocereus polyrhizus*

Phin Yin Sin[1], Suat Hian Tan[1]*, Zhe Cheng Ng[2], Nyuk Ling Ma[3] and Wan Nurul Hidayah Wan Anuar[4]

1. Faculty of Industrial Sciences and Technology, College of Computing and Applied Sciences, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang, Darul Makmur, Malaysia
2. Kota Pharma (M) Sdn Bhd,1,2, &3 Jalan TTC 12, Cheng Industrial Estate,75250, Melaka, Malaysia
3. School of Fundamental Science, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu Darul Iman, Malaysia
4. Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch, Tapah Campus, Tapah Road, Malaysia

*Corresponding author: tshian@umpsa.edu.my

ABSTRACT

Dragon fruit *(Hylocereus polyrhizus)* is a tropical fruit. Recently, it has gained interest from the public due to its potential beneficial effects on health. The acclimatization of micropropagated *Hylocereus polyrhizus* depends on the application of gibberellic acid (GA_3) to increase plant growth. Eggshells are waste materials from industrial sectors, and they are composed of calcium source that is vital for the development of plant shoots and root. The objective of this research is to investigate the effect of different concentrations of GA_3 and eggshell either added individually or in combination on the growth of shoot length and shoot diameter of *H. polyrhizus*. The result showed the shoot length of the *H. polyrhizus* increased by approximately 54.69%, from 0.64 ± 0.13 cm to 0.99 ± 0.26 cm, as the concentration of GA_3 increased from 0 ppm to 10 ppm. Furthermore, this finding also reported that with eggshells, GA_3 showed an adverse effect on the development of shoot diameter. The growth of shoot length and shoot diameter with the addition of eggshell was different, perhaps due to the gibberellic acid affecting the shoot length but not the shoot diameter. Generally, the growth of shoot length and shoot diameter with eggshells was higher in comparison with those without eggshells. With that, we can prove that eggshell is a good additive to promote the growth of *H. polyrhizus*.

Key words: Dragon fruit, eggshell, gibberellic acid, micropropagation

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INTRODUCTION

Dragon fruit or known as pitaya or pitahaya is a promising tropical fruit and is also referred to as the fruit of the genus of *Hylocereus*. Two common species of dragon fruit that can be found in Malaysia are *H. undatus* (white flesh) and *H. polyrhizus* (red flesh). Dragon fruit is widely cultivated in numerous Southeast Asia countries including Thailand, Vietnam, and Malaysia due to their identical tropical climates ideally for propagating a variety of tropical fruits, specifically the dragon fruit. Dragon fruit is gaining increasing interest and the demand for the fruit is extensively rising in the fruit market all over the world. This fame is attributed to the fact that dragon fruit comprises several qualities in terms of its attractive appearance, nutritive value, and health benefits (Ng *et al*., 2021; Wijitra, 2013). The nutritive benefits of dragon fruit for human health can be understood by its rich source of essential nutrients provided for consumers including vitamins, minerals, crude fibers, complex carbohydrates, and valuable antioxidants like phytoalbumins and betacyanin (Hitendraprasad *et al*., 2020). According to Arivalagan *et al*. (2021), dragon fruit contains significant amounts of vitamins and minerals such as sodium, magnesium, potassium, and phosphorus which the researchers claimed to have higher mineral contents in comparison to mangosteen, mango, and pineapple. Moreover, the dragon fruit tree also possesses great potential as an economic crop in extreme conditions like drought, extremely high temperatures, and poor soil (Ng *et al*., 2020).

Apart from that, an increasing number of studies have reported the medicinal value of dragon fruit since it provides protection against some chronic diseases such as cardiovascular disorders and cancers (Tenore *et al*., 2012; Manan *et al*., 2019). This is owing to the presence of antioxidant constituents such as phenolics, vitamin C, anthocyanidin, tannins, and other bioactive compounds that help to scavenge and neutralize free radicals to avoid peroxidation and potential damage transient chemical species (Tan & Rezaul-Karim, 2018). Besides, dragon fruit is also effective in controlling hyperglycemia and it is believed that the positive effect is attributed to the high dietary fiber content in the dragon fruit. Thus, dragon fruit is referred to as one of the medicinal plants with potential for Type 2 diabetes (diabetes mellitus) treatment (Poolsup *et al*., 2019). The mechanism of soluble dietary fiber in dragon fruit lowers the blood glucose level by absorbing the water to form viscous solutions in the digestive tract, thus slowing down the rate of nutrients being absorbed. Other therapeutic properties of dragon fruit include anti-cancer, antimicrobial, and anti-thrombotic effects, as well as preventing dyslipedemia and hypercholesterolemia (Luu *et al*., 2021).

In most case, propagation of dragon fruit tree is done by a conventional method such as seed and cutting, whereby cutting allow the production of plants with identical characteristics to the parent plant. However, the application of conventional methods in controlling the physical and chemical environment for plant growth is time-consuming and is limited to large-scale production of propagating (Bhatia & Sharma, 2015; Borchetia *et al*., 2022). Therefore, plant tissue culture or micropropagation has become a rapid alternative method for the bulk production of pathogen-free plants in a shorter period with minimal application of starting materials (Espinosa-Leal *et al*., 2018). This technique is conducted within a controlled environment where the plant is provided with a sufficient amount of nutrients in growth media (Wan-Anuar *et al*., 2019).

Meanwhile, successful *ex-vitro* acclimatization of micropropagated plantlets is a key process to determine whether the plantlets can successfully grow in a new environment since the plantlets are easily impaired by sudden changes in environmental conditions. The difficulties in transplanting the *in-vitro* plantlets to soil share the common characteristics that are inconsistent development, in terms of transpiration rates when compared to those grown in a greenhouse (Kshitij & Rao, 2012; Abdalla *et al*., 2022). This is caused by the abnormal functioning of stomata and thin cuticles on these micropropagated plantlets and might lead to plant death. Therefore, some plant hormones such as gibberellic acid, ethylene, and abscisic acid have been considered in the adaptive response of plant growth to environmental stress in previous studies (Verelst *et al*., 2010). Gibberellic acid (GA3) promotes cell division and elongation, thereby increasing the height of the plant, number of leaves, and the length of plants (El Khoury *et al*., 2016).

According to Ng *et al*. (2019), food waste is rated as the top three solid wastes in Malaysia. There were plenty of previous studies that reported that food waste can be used as fertilizer for plants. Eggshells are one of the waste materials from most industrial sectors and can be obtained easily from hatcheries, homes, and some fast-food industries. However, their disposal is always a problem since these wastes might contribute to environmental pollution. As reported by Wijaya *et al*. (2019), the hard structure of eggshell is composed of approximately 98.2 % calcium carbonate, making it a good calcium source that is vital for development of shoot tissue and root besides a good soil stabilizing agent (King'ori, 2011). In addition, the eggshell also contains a source of amino acids, uric acid, and sialic acid. As for nutrients, eggshell supplies a variety of macronutrients and micronutrients such as potassium, magnesium, nitrogen, phosphorus, calcium, zinc, and chloride that are vital for plant growth (Ma *et al*., 2019). Thus, we were the first attempt to investigate the combination of eggshell with different concentrations of gibberellic acid to the growth of *H. polyrhizus* in ex-vitro conditions.

MATERIALS AND METHODS

Preparation of plant materials

Sterile plantlets of *H. polyrhizus* were obtained from the plant culture laboratory of Universiti Malaysia Pahang which had been in-vitro cultured for 30 days. The *H. polyrhizus* was removed from the jar carefully and the roots of the plantlet were cleansed gently under running water to remove the attached media.

Preparation of the soils, additives, and hormone

For the preparation of ex-vitro explant, some polystyrene bags were prepared and were filled with conventional soils and organic fertilizers in the ratio of 7:3. The organic fertilizers used were composed of coco peat, burnt soil, river sand, burnt Husk, rich humus, and charcoal powder. A whole dry eggshell weighing approximately 5 grams was crushed using a mortar and pestle and ready to bury into polystyrene bags. Different concentration of gibberellic acid (Merck Millipore, Germany) was prepared (0 ppm, 1 ppm, 10 ppm, and 50 ppm) and stored in different spraying bottles.

Classification of polystyrene bags

The polystyrene bags were categorized into two respective groups: the absence of eggshells and the presence of eggshells. For the absence of eggshell, no eggshell was added; whereas for the presence of eggshells, one whole eggshell was crushed and buried into the polystyrene bag that filled with soils. The *H. polyrhizus* that grown in soil with absence or presence of eggshell were treated with different concentrations of gibberellic acid (0−50 ppm). The control was the *H. polyrhizus* that grown in the soil without any additive or hormone. The effect of eggshell and gibberellic acid on the growth of *H. polyrhizus* was measured in terms of shoot length and shoot diameter, in centimetre (cm).

Micropropagation

The *H. polyrhizus* was then transferred into the middle region of the soil. This step was repeated three times and the initial length of the *H. polyrhizus* was recorded. Different treatments of gibberellic acid were applied on the *H. polyrhizus* twice daily when required. The length of shoots and diameter of shoots were observed and recorded weekly. The percentage of increment of these two parameters was calculated through the following equation.

$$\text{Increment growth}(\%) = \frac{\text{Treatment-Control}}{\text{Control}} \times 100$$

Statistical analysis

The data was analyzed using the one-way ANOVA. The mean values were compared by utilizing Duncan's multiple range test at a 5% (p=0.05) significance level, using the SPSS software version 22 (SPSS Inc. USA).

RESULTS AND DISCUSSION

Effect of gibberellic acid on the shoot length of *H. polyrhizus* without the addition of eggshell

The experiment was conducted with various concentrations of gibberellic acid which were 0 ppm, 1 ppm, 10 ppm, and 50 ppm to determine the effect of gibberellic acid on the growth of the *H. polyrhizus* trees after six weeks.

The growth of the shoot length of the *H. polyrhizus* was observed and recorded in Figure 1. The growth of shoot length increased as the concentration of gibberellic acid used increased from 0 ppm to 10 ppm without the addition of eggshell. In the sixth week, the plantlets showed 0.64 ± 0.13 cm of growth in shoot length at 0 ppm of gibberellic acid while at 1 ppm and 10 ppm of gibberellic acid showed growth of 0.99 ± 0.12 cm and 0.99 ± 0.26 cm respectively. An increment of approximately 54.69% was recorded when gibberellic acid increased from 0 ppm to 10 ppm. However, 50 ppm showed a sign of a reduction in terms of the growth of the shoot length, where the growth of shoot length was 0.77 ± 0.06 cm. This phenomenon happened probably due to the high concentration of gibberellic acid (50 ppm) inhibited the growth of *Hylocereus polyrhizus*, whereas a lower concentration of gibberellic acid (0 ppm, 1 ppm, & 10 ppm) promoted the growth of the *H. polyrhizus*. The finding was in close conformity with the article written by Thakare *et al*. (2011), who reported that a low concentration of gibberellic acid has a positive effect on plants. In contrast, a high concentration of gibberellic acid harmed plants.

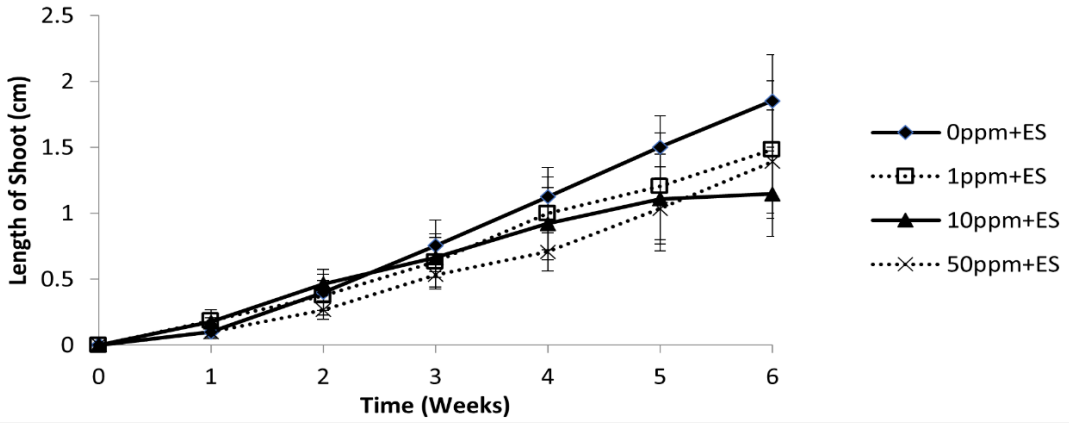

Fig. 1. Effect of different concentrations of GA_3 on the growth of shoot length of *H. polyrhizus* without eggshell after 6 weeks of ex-vitro culture. The results were expressed in mean ± S.E (n=3).

Effect of gibberellic acid on the shoot length of *H. polyrhizus* without the addition of eggshell

Based on Figure 2, in the presence of eggshell, 0 ppm of gibberellic acid showed the highest performance in terms of growth of the shoot length for *H. polyrhizus*, followed by 1 ppm, 10 ppm, and 50 ppm of gibberellic acid with the growth of shoot length of 1.85 ± 0.35 cm, 1.48 ± 0.52 cm, 1.15 ± 0.32 cm and 1.39 ± 0.39 cm respectively. The addition of 1 ppm, 10 ppm, and 50 ppm of gibberellic acid together with eggshells has a significant effect on shoot length as compared to the 0 ppm of gibberellic acid. In addition, it was also observed that in the presence of eggshells, gibberellic acid harmed the development of the shoot length. When the concentration of gibberellic acid is raised, the growth of the shoot length is reduced. This result showed an opposite graph pattern as compared to Figure 1, with the absence of eggshell in its soil. In brief, 0 ppm of gibberellic acid showed the best compatibility with eggshells, and 10 ppm of gibberellic acid had the worst compatibility with eggshells. This situation is probably because the calcium and gibberellic acid are antagonists. This is because gibberellic acid controls the growth extension of plants by inducing the uptake of calcium and removing calcium from the plant wall to permit plant growth enhancement (Hepler & Wayne, 1985). Similar antagonistic situations of calcium application with gibberellic acid on Avena (oat) stem segments and lettuce hypocotyl sections were found (Moll & Jones, 1981; Montague, 1993).

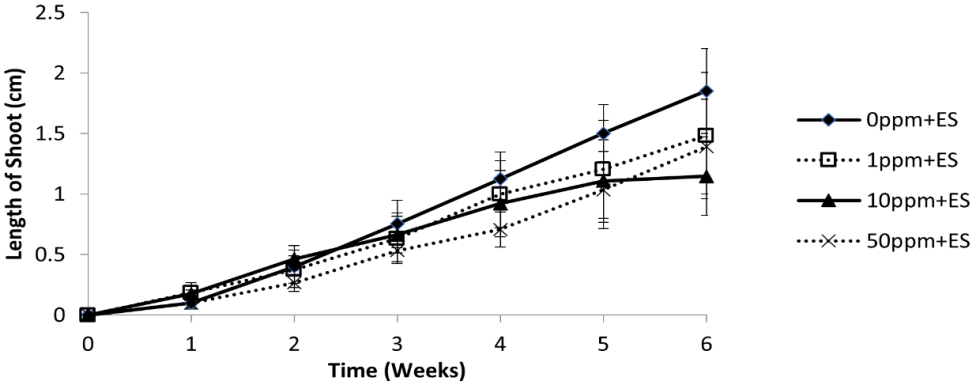

Fig. 2. Effect of different concentrations of GA_3 on the growth of shoot length of *H. polyrhizus* with eggshell (ES) after 6 weeks of ex-vitro culture. The results were expressed in mean ± S.E (*n*=3).

Effect of gibberellic acid on the shoot diameter of *H. polyrhizus* without the addition of eggshell

Based on Figure 3, the growth of diameter of the shoot without eggshells and with 0 ppm, 1 ppm, 10 ppm, and 50 ppm gibberellic acid was 0.11 ± 0.01 cm, 0.09 ± 0.02 cm, 0.14 ± 0.03 cm and 0.09 ± 0.02 cm respectively. It was observed that the growth of shoot diameter without eggshells did not entirely show a similar growth pattern of shoot length without eggshells (Figure 1) although the observations were obtained from the same plants. The concentration of gibberellic acid that showed the least effect in the shoot diameter was 50 ppm (Figure 3) whereas the concentration of gibberellic acid that showed the least effect in the shoot length without eggshells was 0 ppm (Figure1). The reason behind this phenomenon could fall under two reasons. First, gibberellic acid had a significant effect on cell elongation (Bostrack & Struckmeyer, 1967). Therefore, the gibberellic acid affected the shoot length but not the shoot diameter. On the other hand, the genetic properties of *H. polyrhizus* could affect the growth of shoots. As reported by El Khourya *et al*. (2016), numerous conflicting reports suggested that the response of plants toward gibberellic acid might be due to several factors such as the inheritance of plants and the environmental conditions.

Effect of gibberellic acid on the shoot diameter of *H. polyrhizus* with the addition of eggshells

Based on Figure 4, the growth diameter of the shoot with eggshells with 0 ppm, 1 ppm, 10 ppm, and 50 ppm of gibberellic acids was 0.26 ± 0.02 cm, 0.13 ± 0.03 cm, 0.12 ± 0.02 cm, and 0.12 ± 0.02 cm respectively. It was seen that the growth of shoot diameter with the presence of the eggshell declined gradually from 0.26 ± 0.02 cm to 0.12 ± 0.02 cm as the concentration of gibberellic acid increased from 0 ppm to 50 ppm. The situation seemed to occur similarly for Figure 2, with 0 ppm of gibberellic acid showing the best performance in terms of shoot diameter while 10 ppm and 50 ppm showed the least performance. Therefore, it has been proven that gibberellic acid and eggshells have adverse effects on each other. The presence of eggshells and high concentration of gibberellic acid caused the growth to be retarded, especially in the growth of shoot diameter. There was a significant effect when gibberellic acid was applied together with the eggshell for both shoot length and shoot diameter. The antagonistic effect affected the shoot diameter as compared to the shoot length. This might be due to the gibberellic acid controls the elongation of shoot length. When gibberellic acid was applied to grow the *H. polyrhizus*, less amount of calcium was required to support their growth.

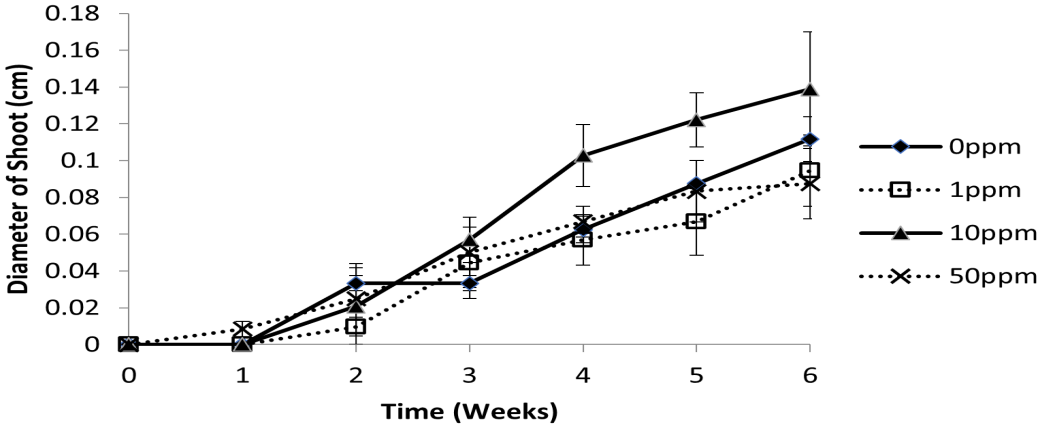

Fig. 3. Effect of different concentrations of GA_3 on the growth of shoot diameter of *H. polyrhizus* without eggshell after 6 weeks of ex-vitro culture. The results were expressed in mean ± S.E (*n*=3).

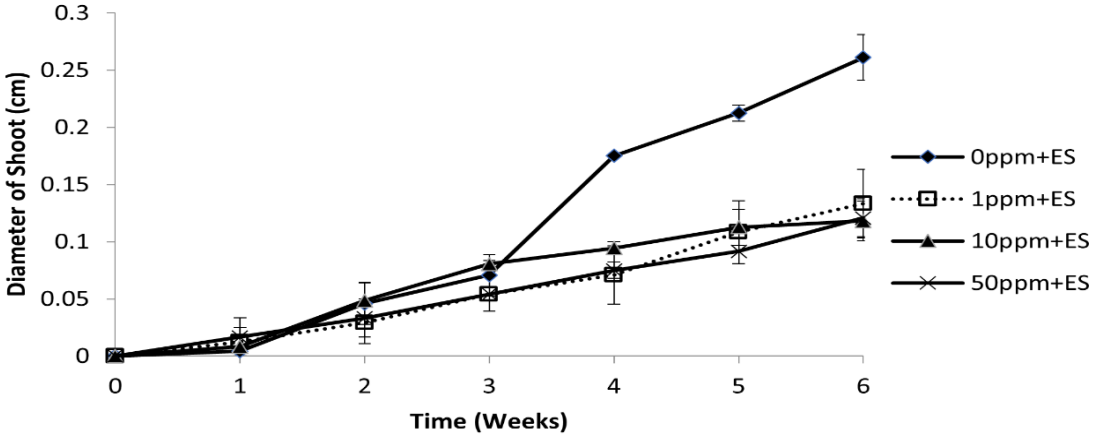

Fig. 4. Effect of different concentrations of gibberellic acid on the growth of shoot diameter of *H. polyrhizus* with eggshell after 6 weeks of ex-vitro culture. The results were expressed in mean ± S.E (*n*=3).

Comparison between shoot length and shoot diameter of *H. polyrhizus*

Based on all four graphs, it could be seen that the growth of shoot length and shoot diameter with eggshell was higher than the growth of shoot length and shoot diameter without eggshell, except for 10 ppm of gibberellic acid in terms of diameter growth because the growth of shoot diameter without eggshell was 0.14 ± 0.03 cm, while the growth of shoot diameter with eggshell was 0.12 ± 0.02 cm. Based on Figure 1, the highest shoot length was recorded at 0.99 ± 0.26 cm with the addition of 10 ppm of gibberellic acid while in Figure 2, the highest shoot length was 1.85 ± 0.35 cm without the addition of gibberellic acid. Besides, in the absence of gibberellic acid, the treatment with eggshell significantly promotes the shoot length of *H. polyrhizus* ($p<0.05$), as shown in Table 1. In the addition of eggshell, the shoot length elevated from 0.64 ± 0.13 cm to 1.85 ± 0.35 cm, an increment of 189% recorded in the condition where 0 ppm of gibberellic acid was added (Figure 3). While the shoot diameter only recorded an increment of 136 %, from 0.11 ± 0.01cm to 0.26 ± 0.02 cm when 0 ppm of gibberellic acid was added (Figure 4).

The reason for the growth increment even without gibberellic acid was due to the ability of eggshells to act as an additive in promoting the growth of *H. polyrhizus*. This is because eggshells contain calcium carbonate and various trace elements such as potassium, magnesium, nitrogen, phosphorus, calcium, zinc, and chloride which are vital for plant growth (Wijaya *et al*., 2019). Besides, the calcium carbonate present in eggshells could be considered one of the natural and inexpensive sources of calcium. Moreover, the presence of the main component, calcium carbonate in the eggshells could promote the growth of *H. polyrhizus* by reducing the acidity of the soil (Khairnar & Nair, 2019). This is achieved when acidic soil reacts with calcium carbonate to form neutral clay, water, carbon dioxide, and aluminium oxide to maintain the optimum pH for the growth of *H. polyrhizus* at pH between pH 5.5 to pH 6.5 (Gunasena *et al*., 2007). The fact behind this statement was that because the pH of the soil will constantly change due to the fertilizer or hormone applied, with the addition of eggshells as a

pH balancing compound, the pH that once acted as a limiting factor in the growth of *H. polyrhizus* could be lessened. Therefore, the application of eggshells as additives has become a new potential method to remediate soil contaminated with heavy metals (Luo *et al*., 2018).

Table 1. Shoot length and shoot diameter of *H. polyrhizus* after 6 weeks

Concentration of Gibberellic acid (GA_3)	Soil without eggshell		Soil with eggshell	
	Shoot length (cm)	Shoot diameter (cm)	Shoot length (cm)	Shoot diameter (cm)
0 ppm	0.64 ± 0.13 ^{ab}	0.11 ± 0.01 ^{a}	1.85 ± 0.35 ^{e}	0.26 ± 0.02 ^{a}
1 ppm	0.99 ± 0.12 ^{bcd}	0.09 ± 0.02 ^{a}	1.48 ± 0.52 ^{de}	0.13 ± 0.03 ^{a}
10 ppm	0.99 ± 0.26 ^{bcd}	0.14 ± 0.03 ^{a}	1.15 ± 0.32 ^{bcd}	0.12 ± 0.02 ^{a}
50 ppm	0.77 ± 0.06 ^{ab}	0.09 ± 0.02 ^{a}	1.39 ± 0.39 ^{cde}	0.12 ± 0.02 ^{a}

Note: values are expressed as mean ± standard deviation. Different superscript letters in the same column indicate significant differences ($p \leq 0.05$).

CONCLUSION

Based on the findings of the present study, it may be concluded that the growth of shoot length of the *H.polyrhizus* increased as the concentration of gibberellic acid increased without the addition of eggshells. Nevertheless, a high concentration of gibberellic acid could hurt the growth of *H.polyrhizus*. Besides, this finding also has proven that the combination of eggshells and gibberellic acid retarded the growth of *H. polyrhizus*. Meanwhile, it is observed that the growth of shoot length and shoot diameter with the addition of eggshells was different, perhaps due to the gibberellic acid affecting the shoot length but not the shoot diameter. Last but not least, the growth of shoot length and shoot diameter with the addition of eggshells was higher in comparison with the growth of shoot length and shoot diameter in the absence of eggshells, probably because of the high calcium source offered by the eggshells. Therefore, it could be inferred that eggshells are a good additive to promote the growth of *H. polyrhizus*.

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ETHICAL STATEMENT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abdalla, N., El-Ramady, H., Seliem, M.K., El-Mahrouk, M.E., Taha, N., Bayoumi, Y., Shalaby, T.A. & Dobránszki, J. 2022. An academic and technical overview on plant micropropagation challenges, Horticulturae, 8: 677. https://doi.org/10.3390/horticulturae8080677

Arivalagan, M., Karunakaran, G., Roy, T.K., Dinsha, M., Sindhu, B.C., Shilpashree, V.M., Satisha, G.C. & Shivashankara, K.S. 2021. Biochemical and nutritional characterization of dragon fruit (*Hylocereus* species). Food Chemistry, 353(2): 12946. https://doi.org/10.1016/j.foodchem.2021.129426

Bhatia, S. & Sharma, K. 2015. Microenvironmentation in micropropagation. In: Modern Applications of Plant Biotechnology in Pharmaceutical Sciences. S. Bhatia, K. Sharma, R. Dahiya & T. Bera (Eds.). Academic Press. pp. 345-360. https://doi.org/https://doi.org/10.1016/B9780-12-802221-4.00010-8

Bostrack, J.M. & Struckmeyer, B.E. 1967. Effect of gibberellic acid on the growth and anatomy of C*oleus Blumei*, *Antirrhinum Majus* and *Salvia Splendens*. New Phytologist, 66(4): 539-544.

Borchetia, A., Neog, M. & Dutta, S. 2022. Review on various regeneration techniques in dragon fruit (*Hylocereus* spp.). International Journal of Plant & Soil Science, 34(24): 323-330. https://doi.org/10.9734/ijpss/2022/v34i242646

Espinosa-Leal, C.A., Puente-Garza, C.A. & García-Lara, S. 2018. In vitro plant tissue culture: means for production of biological active compounds. Planta, 248: 1-18. https://doi.org/10.1007/s00425-018-2910-1

Hepler, P.K. & Wayne, R.O. 1985. Calcium and plant development. Annual Review of Plant Physiology, 36(1): 397-439. https://doi.org/10.1146/annurev.pp.36.060185.002145

Hitendraprasad, P.P. & Hegde, K. 2020. *Hylocereus* spp. (Dragon fruit): A brief review. International Journal of Pharmaceutical Sciences Review and Research, 60(1): 55-57.

Khairnar, M.D. & Nair, S.S. 2019. Study on eggshell and fruit peels as a fertilizer. In: Proceedings of International Conference on Sustainable Development, Novateur Publications, India. pp. 25–27.

El Khourya, R., El Deen, S.N, Boustany, N., Rizk, G.A & Geagea. L. 2016. Effect of different concentrations of gibberellic acid on the growth of *Euphorbia Pulcherrima*. International Journal of Plant, Animal and Environmetal Sciences, 9(1): 54-62. https://doi.org/10.21276/Ijpaes

King'ori, A.M. 2011. A review of the uses of poultry eggshells and shell membranes. International Journal of Poultry Science, 10(11): 908-912. https://doi.org/10.3923/ijps.2011.908.912

Kshitij, K. & Rao, I.U. 2012. Morphophysiologicals problems in acclimatization of micropropagated plants in-*ex vitro* conditions - A reviews. Journal of Ornamental and Horticultural Plants, 2(4): 271-283.

Liaotrakoon, W. 2013. Characterization of dragon fruit (*Hylocereus* spp.) components with valorization potential (PhD). Ghent University, Belgium.

Luo, W., Ji, Y., Qu, L., Dang, Z., Xie, Y., Yang, C., Tao, X., Zhou, J. & Lu, G. 2018. Effects of eggshell addition on calcium-deficient acid soils contaminated with heavy metals. Frontiers of Environmental Science and Engineering, 12(3): 1-10. https://doi.org/10.1007/s11783- 018-1026-y

Luu, T.T. H., Le, T.L., Huynh, N. & Quintela-Alonso, P. 2021. Dragon fruit: A review of health benefits and nutrients and its sustainable development under climate changes in Vietnam. Czech Journal of Food Sciences, 39(2): 71-94. https://doi.org/10.17221/139/2020-CJFS

Manan, E.A., Gani, S.S.A., Zaidan, U.H. & Halmi, M.I.E. 2019. Characterization of antioxidant activities in red dragon fruit (*Hylocereus polyrhizus*) pulp water-based extract. Journal of Advanced Research in Fluid Mechanics and Thermal Sciences, 61(2): 170-180.

Ma, N.L., Khoo, S.C., Loke, L.K., Lam, S.S. & Tan, S.H. 2019. Growth media derived from solid waste for orchid *Dendrobium kingianum* culture. Malaysian Applied Biology, 48(1): 73-78.

Moll, C. & Jones, R.L. 1981. Calcium and gibberellin-induced elongation of lettuce hypocotyl sections. Planta, 152(5): 450-456. https://doi.org/10.1007/BF00385362

Montague, M.J. 1993. Calcium antagonists inhibit sustained gibberellic acid-induced growth of *Avena* (oat) stem segments. Plant Physiology, 101(2): 399-405. https://doi.org/10.1104/pp.101.2.399

Ng, Z.C., Tan, S.H., Mahmud, S.H.R.S. & Ma, N.L. 2020. Preliminary study on micropropagation of *Hylocereus polyrhizus* with waste coconut water and sucrose. Materials Science Forum, 981: 316-321. https://doi.org/10.4028/www.scientific.net/MSF.981.316

Ng, Z.C., Shiekh-Mahmud, S.H.R. & Tan, S.H. 2021. Effects of cytokinin in enhancing the multiplication of vegetative *Hylocereus polyrhizus*. Materials Science Forum, 1025: 97-103.

Poolsup, N., Suksomboon, N., Kurnianta, P.D.M. & Deawjaroen, K. 2019. Effects of curcumin on glycemic control and lipid profile in prediabetes and type 2 diabetes mellitus: A systematic review and meta-analysis. PLoS ONE, 12(9): 1-12. https://doi.org/10.1371/journal.pone.0215840

Tan, S.H. & Karim, M.R 2018. Effects of three drying treatments on the polyphenol content, antioxidant and antimicrobial properties of *Syzygium aromaticum* extract. Chiang Mai Journal of Science, 45(2): 937-948.

Tenore, G.C., Novellino, E. & Basile, A. 2012. Nutraceutical potential and antioxidant benefits of red pitaya (*Hylocereus polyrhizus*) extracts. Journal of Functional Foods, 4(1): 129-136. https://doi.org/10.1016/j.jff.2011.09.003

Thakare, U., Patil, N. & Malpathak, N. 2011. Performance of chick pea under the influence of gibberellic acid and oxygenated peptone during germination. Advances in Bioscience and Biotechnology, 02(01): 40-45. https://doi.org/10.4236/abb.2011.21007

Verelst, W., Skirycz, A. & Inzé, D. 2010. Abscisic acid, ethylene and gibberellic acid act at different developmental stages to instruct the adaptation of young leaves to stress. Plant Signaling and Behavior, 5(4): 473-475. https://doi.org/10.4161/psb.5.4.11421

Wan Anuar, W.N.H., Tan, S.H., Shiekh Mahmud, S.H.R., Mior Norazmi, W.N.B. & Engku Safruddin, E.F.S. 2019. Enhancement effect of BAP and spent mushroom compost in micro-propagation of Sabah snake grass. American Journal of Biochemistry and Biotechnology, 15(4): 190-197. https://doi.org/10.3844/ajbbsp.2019.190.197

Wijaya, V.T. & Teo, S.S. 2019. Evaluation of eggshell as organic fertilizer on sweet basil. International Journal of Sustainable Agricultural Research, 6(2): 79-86. https://doi.org/10.18488/journal.70.2019.62.79.86