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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(SI-2):358-368. **OPEN ACCESS**

The effect of plant growth regulator, Media strength and carbon Sources on *in vitro* seed germination of *Hylocereus undatus* (White Dragon Fruit)

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White dragon fruit (*Hylocereus undatus*) has become an important commodity in Malaysia due to its health benefits and ornamental purpose. Dragon fruit are susceptible to diseases like many other fruits particularly using conventional production method. This study investigates the suitable conditions for *in vitro* culture of *H. undatus* as conventional propagation has failed to meet market demand. The seeds were germinated in Murashige and Skoog (MS) basal medium consisted of different plant growth regulators (PGRs), MS of different strength and MS supplemented with different carbon sources as factors affecting germination. The time taken for the seeds to germinate, percentage of germination, shoot height, and root length were chosen as parameters to study the effects of those factors. In all treatments, the germination rate was 100%. It was found that *H. undatus* seeds germinate fastest in MS + 1 mg/L BAP with the lowest mean of day to germinate 5.00 ± 1.79 days but the growth of shoot and root is higher in MSO which is 1.94 ± 0.30 cm and 2.64 ± 0.97 cm. In terms of media strength, *H. undatus* seeds germinated faster in the quarter strength of MS with 5.00 ± 1.30 days compared to the other media strength. Also, the growth of the shoot and root of the explants is optimum in the quarter strength of MS with the highest mean 2.70 ± 0.45 cm and 3.70 ± 0.45 cm. The observation from carbon sources requirement highlighted that seed germination of *H. undatus* was the most optimum in MS with 15 g/L of sucrose with the lowest mean of the germination day is 4 ± 0.00 days. Similarly, the optimum growth of shoot is 2.30 ± 0.57 cm in the same treatment. There is no significant difference between the root length in MS with 15g/L of sucrose and root length in MS with 30g/L of glucose. It is shows that the root of *H. undatus* proliferated and grown in optimum rate in the MS with 15g/L of sucrose and root length in MS with 30g/L of glucose. Optimization of these media is important aspect in this study to ensure the high numbers of seedlings can be produced with the optimum rate of the growth. The study concludes that the *H. undatus* seed required MS culture media without PGR, of quarter strength and supplemented with 15 g/L of sucrose. This culture condition is proposed to be used by tissue culturist to produce seedlings of *H. undatus* to ensure the production will meet the market demand in Malaysia.

Keywords: White Dragon Fruit, Plant Growth Regulator, Carbon Source, Tissue Culture

INTRODUCTION

Dragon fruits are a promising tropical fruit grown in various tropical and subtropical regions such as Southeast Asia, Central, and South America. As a result, there is an increasing appetite for dragon fruit, and the fruit can be found on almost every

tropical fruit market today (Liaotrakoon et al. 2013). Dragon fruit is a healthy and exotic fruit in Malaysia's fruit industry. While it was not indigenous in this country (Hafiz et al. 2019), it has speedy growth and humid resistance. Furthermore, dragon fruits are high in vitamins and minerals, which can aid in the improvement of the human body's metabolism. It is excellent for the digestion and circulation of our blood. Reports showed that the dragon's fruit had a beneficial effect on lowering hypertension and bodily toxins (Hafiz et al. 2019; Zanudin, 2007).

Dragon fruit is traditionally propagated by seeds, cuttings, and grafting (Vishnupriya et al. 2019), but micro propagation has also been used. Although numerous research has explored these general propagation methods of dragon fruit, no widely available information presently exists on the protocols for creating high-quality planting material produced using the tissue culture system (Dahanayake and Ranawake, 2012). The alternate method of rapidly multiplying plants under temperature, humidity, and light conditions is micropropagation or *in vitro* culture to improve pests without any disease. It is, therefore, quicker and more effective than traditional methods of propagation of plants (Thinesh and Seran, 2015). Tissue culture is a form of propagation using controlled nutrients and the climate.

According to Zainudin and Hafiz (2014), the dragon fruit has gained popularity in Malaysia owing to its health benefits and high nutrient content. The production of this fruit decrease because of the outbreak of soft rot caused by *Xanthomonas compestri*. To increase the production of *H. undatus* in Malaysia, the seedling's production needs well-established procedures that are efficient and accurate. In addition, the techniques should produce maximum seedlings with an optimum amount of inputs. However, there is currently no widely available information on a systematic set of protocols to establish *in vitro* seed germination *of H. undatus* done. Many Malaysian institutes report that the development of dragon fruit is minimal. The second study said that dragon fruit cultivation techniques, including stem cutting and grafting, could not keep up with the increased demand (Gunasena et al. 2006). Unfortunately, the complete *in vitro* culture methods do not exist for this genus. This research seeks to determine if various plant regulators and carbon sources have a more significant impact on plant production of shoots and roots for commercial use.

MATERIALS AND METHODS

Plant materials

Seeds of *H. undatus* were obtained from a new and mature fruit purchased at a local Giant supermarket in Jerteh, Terengganu. The fruit originates in Malaysia. The fruit was quickly processed following purchasing.

Sterilization of the seed

Freshly extracted seeds were surface sterilized by dipping in 70% ethanol for two minutes, soaking in a mixture of 1% Clorox added with two drops of Tween 20, and then rinsing three times in distilled water. The sterile seeds was blotted and dried with filter papers (Taski-Ajdukovic and Vasic, 2005).

Plant Growth Hormones (PGRs) Media Preparation

The MS medium (Murashige and Skoog, 1962) was prepared, and PGRs were added. For each treatment, 500 mL of MS medium was prepared; the macro solution, micro solution, Fe EDTA, vitamins, sucrose, PGRs, and Gelrite™ were added in the sequence stated in Table 1. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 minutes. Then, 20 mL of each medium was placed into a test tube. Each treatment had five replicates grown. Each test tube had a single dragon fruit seed grown on MS medium, resulting in a total of 20 replicates for all PGRs treatments. Cultures were grown on a rack in a growth environment with a 16-hour photoperiod and fluorescent light at 25 2°C.

Preparation of Media using different of Media strengths

The MS medium (Murashige and Skoog, 1962) was prepared and supplemented with PGRs. To prepare 500 ml of MS medium for each treatment, the macro solution, micro solution, Fe EDTA, and vitamin, sucrose, PGR, and Gelrite™ were added in the order listed in the Table 2. Before autoclaving at 121°C for 15 minutes, the pH of the medium was adjusted to 5.8. Then, each media was poured into a test tube in a volume of 20 mL.

Table 1: Preparation of 500 ml MS media with different PGRs

No	Stock/ingredient	Stock concentration	TP0 (Control)	TP1 (MS+ 1mg/L BAP)	TP2 (MS+ 2mg/L BAP)	TP3 (MS+ 15mg/L GA)
1	Macro solution	10x	50 ml	50 ml	50 ml	50 ml
2	Micro solution	100x	5 ml	5 ml	5 ml	5 ml
3	Fe EDTA	100x	5 ml	5 ml	5 ml	5 ml
4	Vitamins	100x	5 ml	5 ml	5 ml	5 ml
5	PGRs	100x	-	1 ml	2ml	15ml
6	Sucrose	-	15 g	15 g	15 g	15 g
7	Gelrite (3 g/L)	-	1.5 g	1.5 g	1.5 g	1.5 g

Table 2: Preparation of 500 ml MS media with different strength

No	Stock/ingredient	Stock concentration	TMS0 (Control)	TMS1 (Half strength MS)	TMS2 (Quarter strength MS)	TMS3 (Eighth strength MS)
1	Macro solution	10x	50 ml	25 ml	12.5 ml	6.25 ml
2	Micro solution	100x	5 ml	2.5 ml	1.25 ml	0.625 ml
3	Fe EDTA	100x	5 ml	2.5 ml	1.25 ml	0.625 ml
4	Vitamins	100x	5 ml	2.5 ml	1.25 ml	0.625 ml
5	Sucrose (30 g/L)	-	15 g	15 g	15 g	15 g
6	Gelrite™ (3 g/L)	-	1.5 g	1.5 g	1.5 g	1.5 g

The medium was supplied with various concentrations of basal media according to the treatments listed in Table 2. The items were then disinfected by autoclaving at 121^{o}C for 15 minutes. Five seeds were grown on the MS medium in five test tubes for each concentration. Cultures were kept on a rack in a growth environment with a 16-hour photoperiod and fluorescent light at 25 ± 2^{o}C

Preparation of Media using different Carbon Sources

The MS medium (Murashige and Skoog, 1962) with different carbon sources was prepared. According to Table 3 for preparation of 500ml MS medium. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 15 minutes. Each of the media was poured 20 mL per test tube. For each concentration, a seed was cultured on the MS medium in a test tube. Each of the treatments includes five replicates. Cultures were maintained on a rack in a growth room with 16-hour photoperiod under fluorescent light at 25 ± 2^{o}C.

Treatments with different PGRs on seed germination of H. undatus

Explant of H. undatus was inoculated on MS medium with various PGRs. For seed germination, the medium consisted of MS basal salts, iron source, vitamins, sucrose (30 g/L), and Gelrite ™ (3g/L). Various PGRs were added to the medium. Until autoclaving, the pH was set to 5.8.

Treatments with different strength of media on seed germination of *H. undatus*

MS media was used to inoculate explants. The medium contained MS basal salts, iron source, vitamins, sucrose (30g/L), and GelriteTM (3g/L) with varying media strengths; full media as control, half media, quarter strength, and eighth strength.

Culture Conditions

All media have been sterilized in an autoclave at 121^{o}C for 15 minutes. Murashige and Skoog's single culture tube containing a seed inoculated on MS media Murashige & Skoog (1962). Each treatment was replicated 5 times. The culture was maintained on a rack in the inoculation room at a humidity level of 60% and a photoperiod of 16 hours under fluorescent light at a temperature of 25± 2°C to enable the plants to acclimate to their new environmental conditions.

Data analysis

The cultures were observed periodically and records of studies were made based on visual observation and morphological changes of seedlings and number of shoots and roots. The average shoot and root length were observed and recorded every day for 32 days using rulers.

Statistical Analysis

The percentage of seed germination, the day to germinate, the height of the shoot, and the length of the root were all gathered in this research utilizing a totally randomized design methodology (CRD). The data were analyzed using One Way Analysis of Variance (ANOVA) and Social Science Statistical Software 20 (SPSS) to determine whether there were any statistically significant differences in the means of the two groups. Means that varied substantially were compared using a Multiple Comparisons Table with a Post Hoc Test (Turkey) at a 5% level of significance to ascertain the source of the discrepancies.

Table 3: Preparation of 500 ml MS media with different carbon sources

No	Stock/ ingredient	TCS0 (Control)	TCS1 (15g/L sucrose)	TCS2 (30g/L Fructose)	TCS3 (15g/L Fructose)	TCS4 (30g/L Glucose)	TCS5 (15g/L Glucose)
1	**Macro solution**	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml
2	**Micro solution**	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
3.	**Fe EDTA**	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
4	**Vitamins**	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
5	**Sucrose**	15 g	7.5 g	-	-	-	-
6	**Fructose**	-	-	15 g	7.5 g	-	-
7	**Glucose**	-	-	-	-	15 g	7.5 g
8	**Gelrite™**	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g	1.5 g

Table 4: Treatment with different PGRs

NO	Treatment	Media
1	TP0	MSO
2	TP1	MS+1 mg/L BAP
3	TP2	MS+2 mg/L BAP
4	TP3	MS+15 mg/L GA

RESULTS AND DISCUSSION

For large-scale plant multiplication, plant tissue culture is widely utilized. Furthermore, plant tissue culture technologies have lately gained popularity in research, disease management, plant propagation and enhancement, and phytochemical production. Thousands of microscopic bits of tissue (called explants) are used to create thousands of plants by cloning and growing in controlled settings, regardless of season or weather.

The effect of treatment with PGRs on the day of *H. undatus* germinates

The present research examined seed germination to determine the effect of PGR on the day to germinate. As indicated in Table 5 and Fig 1 below, TP1 germinates earliest at 5 ± 1.79 days, followed by TP0 and TP2 at 8 ± 1.58 days, and the longest was observed in TP3, which is MS with 15 mg/L at 11 ± 4.15 days.

Table 5: Germination time of *H. undatus* seeds treated with different PGRs. Mean with asterisk shows significant difference at p<0.05, n=5

Treatment	Day to germinate (Day) (mean ± SD)
TP0	8 ± 1.10
TP1	5 ± 1.79*
TP2	8 ± 1.58
TP3	11 ± 4.15*

There is a significant difference between the PGR and day to germinate which is proven that the p-value is <0.05. In general, *H. undatus* seeds treated with different plant growth hormones have the same day of germination except in TP1 and TP3. The TP1 required 5 ± 1.79 days to germinate while TP3 required 11 ± 4.15 days to germinate. Winson et al. (2016) also reported that the seed germination of *Hylocereus costaricensis* treatment G1 (MS + 1 mg/L BAP) recorded the highest germination percentage at 93.33% and required 5 days to germinate. However, in this experiment, the explant is inoculated under a 16-hour photoperiod under fluorescent light. It causes a delay in the germination of the seeds in TP3, which is MS in 15 mg/L GA. GA is also used to increase the seed germination percentage. But under light conditions, GA at higher concentration may inhibit the germination potential of some cactus species. Temperature below 12°C and above 28 °C does not favor germination. There are also some inhibitors present in seed testa and also in fruit flesh to maintain the seed dormancy until the favorable condition persists for seed germination (Mariana Rojas-Arechiga, 2000).

The effect of treatment with PGR on shoot height of *H. undatus*

The currents findings revealed that TP0 at 1.94 ± 0.30 cm followed by TP3 at 1.30 ± 0.45 cm, TP1 at 1.00 ± 0.00 cm, and the shortest in TP2 at 0.90 ± 0.22 cm as shown in Fig 2 and Table 6 below. In general, *H. undatus* seeds treated with different plant growth hormones have significantly different shoot heights produced in all of the treatments. In the general *H. undatus* seed treated with plant growth hormones have the same root length produced in all the treatment the p-value is more than 0.05.

Table 6: Shoot height of *H. undatus* treated with different PGRs. Means with asterisk shows significant difference at p<0.05, n=5

Treatment	Shoot height (cm) (mean ± SD)
TP0	1.94 ± 0.30*
TP1	1.00 ± 0.00*
TP2	0.90 ± 0.22*
TP3	1.30 ± 0.45*

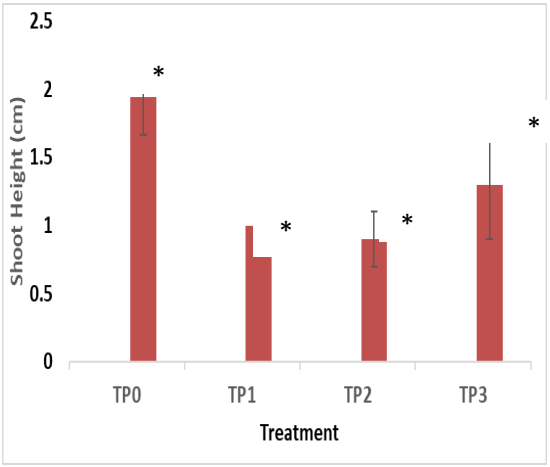

Figure 2: Shoot height of *H. undatus* treated with different PGRs

An increasing level of BAP concentrations showed declining in the number of shoots. The elevation of BAP level contributed to the declining in the formation of multiple shoots, thus reduces their number obtained (Paul et al. 2012). In the study of Elias et al. (2015), the highest mean number of shoots per explant was 3.07 observed in treatment with a combination of 2.0 mg/L NAA and 1.5 mg/L BAP. This result verified that MS medium supplemented with the combination of hormones of 2.0 mg/L NAA and 1.5 mg/L BAP was the optimum medium for shoot formation of this species. However, in this study, there is no combination of PGR was used, which resulting in the production of a higher mean shoot produced in MSO. The NAA has synthetically produced auxin. George (2000) stated that NAA is generally used in plant cell culture at a concentration range of 0.01-1.0 mg/L to induce rooting. It is suggested to add the NAA with the combination with BAP to optimize the growth of explants for in vitro of production of the Cactaceae family.

The effect of treatments with PGR on root length

The findings on root length revealed that TP0 has the highest length at 2.64 ± 0.97 cm, followed by TP2 at 1.70 ± 0.67 cm, TP3 at 1.00 ± 0.45 cm, and the shortest in TP1 at 0.50 ± 0.42 cm Table 7 and Fig 3. There is no significant difference

Table 7: Root length of *H. undatus* treated with different PGRs.

Treatment	Root length (cm) (mean ± SD)
TP0	2.64 ± 0.97
TP1	0.50 ± 0.42
TP2	1.70 ± 0.67
TP3	1.00 ± 0.45

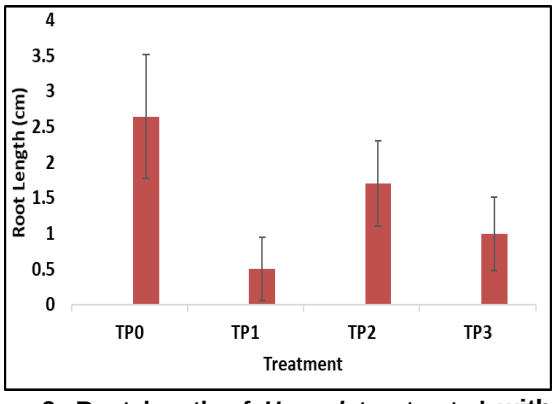

Figure 3: Root length of *H. undatus* treated with different PGRs.

Serrano and da Silva, (2008) reported that *Ferocactus acanthodes* produced 6.6 shoots per explant rooted with MS media lacking PGR. The production of shoot and root is highest in MSO because non-treated media favor seed germination. The presence of PGRs in the media is causes toxicity to the explants. Hence, germination may occur even when no PGRs are added to the culture medium. The plant growth regulators may stimulate endophytic contamination, which competes with micro shoots for nutrients, lower the regeneration potential, inhibit rooting, and may thereby lead to plantlet death (Vidican et al. 2009)

The best media condition of *H. undatus* seeds to germinate is in MS + 1 mg/L BAP with the lowest mean of day to germinate 5 ± 1.79 days, but the growth of shoot and root is higher in MSO, which is 1.94 ± 0.30 cm and 2.64 ± 0.97 cm. This shows that PGR at the first stage of seed germination supported the germination process. Still, at the final stage of the germination process, the PGR is causing inhibitory factors to the explants, resulting in optimum growth observed in shoot and root in non-treated MS, MSO. Based on the other reports, the presence of PGR would cause endophytic contamination to the explants. Therefore, the growth of the explants was also disrupted.

The effect of treatments with media strength on seed germination of *H. undatus*

In this experiment, the seed germination rate is determined by the percentage of seed germination, day to germinate, shoot height, and root length after 32 days. Lema-Rumińska et al. (2012) reported that the best germination medium contained half a number of MS microelements recommended for *Astrophytum asterias.* In contrast, a full-strength MS medium caused a one-week delay in germination. Thus, the inhibitory effect of a full-strength MS medium was not observed with *Turbinicarpus laui* (Rosas et al. 2001). The effect of different media strength on day to germinate in this study revealed that the shortest was found in the TMS2 at 8 ± 1.10 days followed by TMS1 at 6 ± 1.82 days, TMS0 at 8 ± 1.10 days, and the longest in TMS3 at 9 ± 2.28 days Table 8 and Fig 4 below. There is significant difference between the media strength and shoot height which is proven that the p-value is less than 0.05. Thus, in general, *H. undatus* seeds treated with different plant growth hormones have the same day of germination except in TMS1 and TMS2. Seeds in TMS1 required 6 ± 1.82 days to germinate, while seeds in TMS2 required 8 ± 1.10 days to germinate

Table 8: Germination time of *H. undatus* seeds treated with different media strength.

Treatment	Day to germinate (Day) (mean ± SD)
TMS0	8 ± 1.10
TMS1	6 ± 1.82*
TMS2	5 ± 1.30*
TMS3	9 ± 2.28*

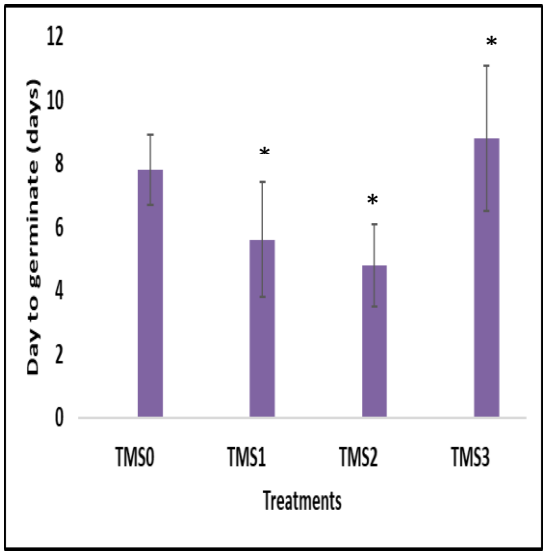

Figure 4: Germination time of *H.undatus* seeds treated with different media strength.

Many studies have reported that half- or even quarter-strength MS, provides a better effect in germination and subsequent early development than full-strength one (Koné et al. 2015, Manzur et al. 2013, Xu et al. 2007). This is possibly attributable to harmful effect of the salts present in the MS formulation (Monnier, 1995). According to Paul et al. (2012), full-strength MS is recommended for later seedling stages when seedlings increase their size and become photosynthetically active. This is because their micro and macronutrient requirements are higher. Bo et al. (2010) reported the highest germination frequency of *P. villosum* var. densissimum in the quarter- and half-strength MS treatments.

The effect of treatments with media strength on shoot height of *H. undatus.*

From these findings shoot height after 32 days recorded TMS2, as the tallest at 2.70 ± 0.45 cm followed by TMS1 at 2.32 ± 0.41 cm, TMS0 at 1.94 ± 0.30 cm, and the shortest in TMS3 at 1.66 ± 0.42 cm Table 9 and Fig 5. There is significant difference between the media strength and shoot height. In general, *H. undatus* seeds treated with different MS strengths have the same shoot height produce except in TMS0, TMS1, and TMS2.

Table 9: Shoot height of *H. undatus* seeds treated with different media strength.

Treatment	Shoot height (cm) (mean ± SD)
TMS0	1.94 ± 0.30*
TMS1	2.32 ± 0.41
TMS2	2.70 ± 0.45*
TMS3	1.66 ± 0.42*

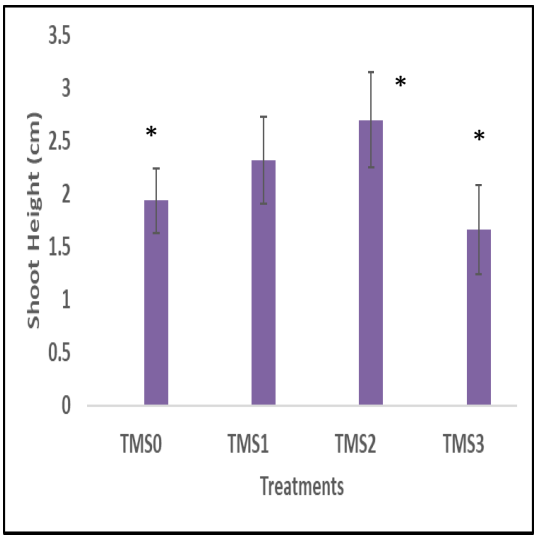

Figure 5: Shoot height of *H. undatus* seeds treated with different media strength.

This study shows that the growth of explants is higher in quarter strength of MS compared to the full, half, and eighth strength of MS. According to Manzur et al. (2013), the full-strength MS level could be slightly toxic for explant and decrease their efficiency growth rates. The quarter strength each refers to the MS composition that reduced into quarter from the full strength of MS. The macronutrient, micronutrient, Fe EDTA, and vitamin are reduced into the quarter, but the growth of explants is higher. The half medium is still caused toxicity and delayed the growing process. The eighth strength has the lowest shoot height is because of the least component of the MS, which leads to deficiencies in nutrient supply to the explant.

The effect of treatments with media strength on root length of *H. undatus*

The length of the root after 32 days as shown

in Table 10 and Fig 6 the longest recorded in TMS2, at 3.70 ± 0.45 cm followed by TMS0 at 2.64 ± 0.97 cm, TMS1 at 2.16 ± 1.19 cm, and the shortest in TMS3 at 2.00 ± 0.50 cm. There is no significant difference between the media strength and root length.

Table 10: Root length of *H. undatus* seeds treated with different media strength.

Treatment	Root length (cm) (mean ± SD)
TMS0	2.64 ± 0.97
TMS1	2.16 ± 1.19*
TMS2	3.70 ± 0.45*
TMS3	2.00 ± 0.50*

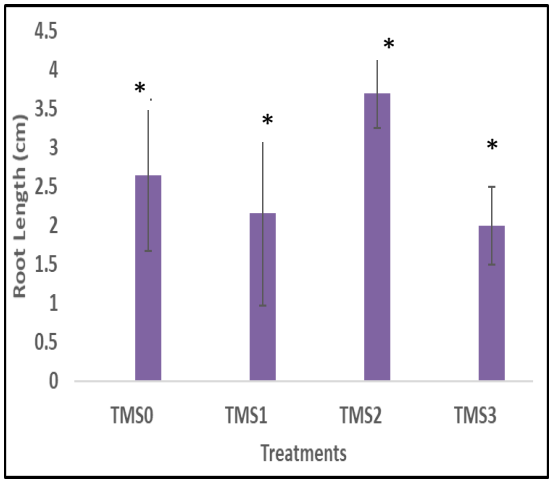

Figure 6: Root length of seeds treated with different media strength. Mean with asterisk shows significant difference at p<0.05, n=5

The root of *H. undatus* seeds is proliferated optimally in the quarter strength of MS with the highest mean 3.70 ± 0.45 cm. This shows that the germination process of explant only required the minimum amount of MS composition. Thus, it helps in reducing production costs for large-scale production of *H. undatus* seedlings.

The effect of treatments with carbon sources on seed germination of *H. undatus*

According to Mosaleeyanon et al. (2004), the impact of the forms and concentrations of various carbohydrates on the growth and production of *in vitro* cultures are still important issues in micropropagation studies. Thus, this research is done to observe the impact of seed germination of *H. undatus* in various carbon sources. As shown in Table 11 and Fig 7 below TCS1, recorded the shortest at 4.00 ± 0.00 days followed by TCS3 at 6.00 ± 3.46 days, TCS5 at 6.20 ± 2.49 days, TCS4 at 6.60 ± 1.52 days, TCS2 at 7.60 ± 0.55 days and the longest in TCS0 at 7.80 ± 1.10 days. This shows no significant difference between the carbon sources and the day of germination. Therefore, *H. undatus* seeds treated with different carbon sources have the same day of germination except in TCS0 and TCS1.

Table 11: Germination time of *H. undatus* seeds treated with different carbon sources. Means with asterisk shows significant difference at p<0.05, n=5

Treatment	Day to germinate (Day) (mean ± SD)
TCS0	8 ± 1.10*
TCS1	4 ± 0.00*
TCS2	8 ± 0.55
TCS3	8 ± 3.46
TCS4	7 ± 1.52
TCS5	6 ± 2.49

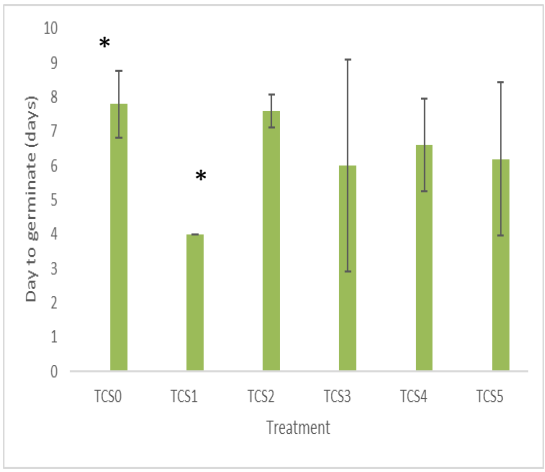

Figure 7: Germination time of *H. undatus* seeds treated with different carbon sources. Means with asterisk shows significant difference at p<0.05, n=5

Isah and Umar (2017) also stated that the carbon source experiment revealed sucrose outperformed glucose, fructose, and maltose *in vitro* synthesis. However, there is no statistically significant difference between the day to germinate in MS with 30g/L sucrose and the day to germinate in MS with 30g/L fructose. Thus, it is shown that the seeds of *H. undatus* germinate at the fastest rate in the MS containing 30g/L sucrose.

The effect of treatments with carbon sources on shoot height of *H. undatus*

Table 12 and Fig 8, the height of the shoot

after 32 days TCS1, recorded the highest at 2.30 ± 0.57 cm, followed by TCS4 2.06 ± 0.26 cm, TCS0 1.94 ± 0.30 cm. TCS5 at 1.90 ± 0.55 cm, TCS3 at 1.66 ± 0.32 cm, and the shortest in TCS2 at 1.60 ± 0.42 cm. No significant difference between the carbon sources and shoot height. *H. undatus* seeds treated with different carbon sources have different shoot heights produced in all treatments.

According to Xiong and Wu (2003), the growth and development of in vitro grown plants depend on factors such as macro-and micro-elements composition, carbon source, and plant growth regulator. However, the data obtained that the carbon sources do not significantly affect the shoot production. This outcome is might cause by a long gap time of harvesting to the seeds extracting. Therefore, the seeds might lose viability as they do not process directly from harvesting.

Table 12: Shoot height of *H. undatus* seeds treated with different carbon sources. Means with asterisk shows significant difference at p<0.05, n=5

Treatmen	Shoot height (cm) (mean ± SD)
TCS0	1.94 ± 0.30
TCS1	2.30 ± 0.57
TCS2	1.60 ± 0.42
TCS3	1.66 ± 0.32
TCS4	2.06 ± 0.26
TCS5	1.90 ± 0.55

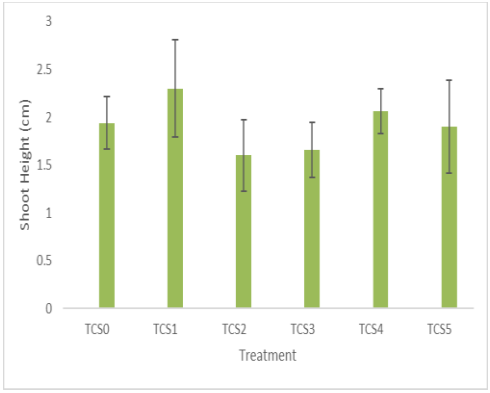

Figure 8: Shoot height of *Hylocereus undatus* seeds treated with different carbon sources. Means with asterisk shows significant difference at p<0.05, n=5

The effect of treatments with carbon sources on root length of *H. undatus*

Table 13 and Fig 4.9, the root of the root after 32 days. The longest were recorded in the TCS4, at 1.30 0.27 cm, followed by TCS1 at 3.00 0.50 cm, TCS0 and TCS5 at 2.64 0.84 cm, TCS2 at 1.76 0.73 cm, and TCS3 at 1.30 0.27 cm. There is significant difference between carbon sources and root length, with a p-value less than 0.05. Except for TCS1, TCS3, and TCS4, *H. undatus* seeds treated with various carbon sources generate the same root length.

However, there is no significant difference in root length between MS with 15g/L sucrose and MS with 30g/L glucose. The root of *H. undatus* is demonstrated to multiply and expand at an optimal rate in MS with 15g/L sucrose and MS with 30g/L glucose. Sucrose is the most abundant sugar in the endosperm early in germination, whereas glucose predominates later on, and fructose is the last (Aoki et al. 2006). According to Vespasiano and Wagner (2003), osmoticum influenced embryogenesis and plant regeneration, whereas sucrose enhanced root development at all concentrations. However, increasing the concentrations of sucrose (0.2-0.3 M) and sorbitol (0.3 M) might increase the synthesis of phenolic compounds. It demonstrates the study's finding that the root develops the fastest in TCS1, which is MS in 15 g/L sucrose compared to TCS0. As a result of a phenolic component, TCS0, which is MS with 30 g/L sucrose, will limit root development.

Table 13: Root length of *H. undatus* seeds treated with different carbon sources. Means with asterisk shows significant difference at p<0.05, n=5

Treatment	Root length (cm) (mean ± SD)
TCS0	**2.64 ± 0.97**
TCS1	**3.00 ± 0.50***
TCS2	**1.76 ± 0.73**
TCS3	**1.30 ± 0.27***
TCS4	**3.10 ± 0.65***
TCS5	**2.64 ± 0.84**

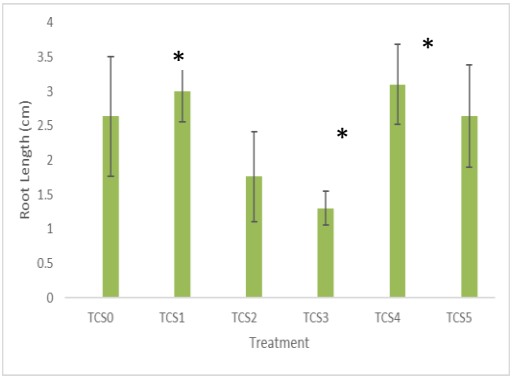

Figure 9: Root length of *H. undatus* seeds treated with different carbon sources. Means with asterisk shows significant difference at p<0.05, n=5

General observations

Generally, all seeds germinated. On day 8 the seeds began to sprout and day 12 was when shoots appeared. It began to grow roots on day 16, cactus spines on day 20. On day 24, shoots and roots became longer and on day 32, the areole is elevated. Day 40, the balls have completely formed and the roots also progress deeper into the agar.

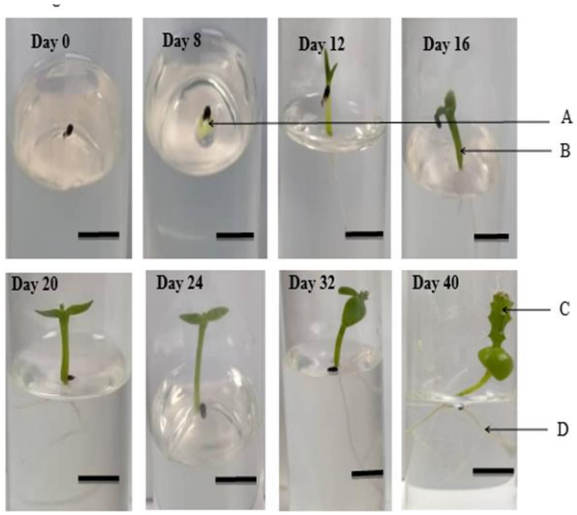

Figure 10: Seedlings from day 0 until day 40. (A) a shoot produced. (B) stem. (C) shoot specialized to form areole. (D) roots. The bar indicates 1 cm.

CONCLUSION

Plant growth regulators, media strength, and carbon sources are critical in ensuring optimal development and germination. This research studied the following in *H. undatus* seed. The most optimal medium condition for seeds to germinate was MS + 1 mg/L BAP with the lowest mean of day to germinate 5 ± 1.79 days. However, shoot and root development are more excellent in MSO at 1.94 ± 0.30 cm and 2.64 ± 0.97 cm. This shows that the presence of PGR during the early stages of seed germination helped germination, but that later in the germination process, the PGR became an inhibitory factor to the explants, resulting in optimal development seen in shoot and root in the control medium.

Concerning media strength, *H. undatus* germinated more quickly in the quarter strength of MS with 5 ± 1,30 days compared with other media strengths. Thus, shoot and root development occur to its greatest extent in the MS quarter when it is 2.70 cm and 3.45 cm from the base. This germination phase allows just the smallest quantity of MS to grow. So, it assists in the large-scale manufacturing of the Seed.

The study concludes that the *H. undatus* seed required culture media without PGR of quarter strength and supplemented with 15 g/L of sucrose. Therefore, tissue culturists propose this culture condition to produce seedlings of *H. undatus* to ensure the product will meet the market demand in Malaysia.

ACKNOWLEGEMENT

The whole Faculty of Bioresources and Food Industry (FBIM). University Sultan Zainal Abidin Malaysia Besut campus staff and laboratory technicians were recognized for their contributions and advice during the project.

CONFLICT OF INTEREST

The authors declare no complicit of interest regarding this manuscript

AUTHOR CONTRIBUTIONS

NHM and TAA designed and supervised the experiments. AAL, wrote the manuscript and reviewed the manuscript. NBS and SFK performed experiment and the treatments.

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