



In vitro Regeneration of Red Dragon Fruit (*Hylocereus polyrhizus*) Stem Segments

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Abstract: Traditionally, the propagation of dragon fruit (*Hylocereus* spp.) is carried out through seed or vegetative cuttings. However, propagation by cuttings is too slow to meet the demand for large-scale plantations and is not genetically true-to-type. With the fruits' increasing demand, an effective *in vitro* propagation of dragon fruit was developed to produce high – quality planting materials for large-scale production of dragon fruit. This study was conducted to establish the appropriate concentration of bleach for surface sterilization and determine the effective level of 6-benzylaminopurine (BAP) for the shoot initiation of dragon fruit explants *in vitro*. Surface sterilization of the explants using commercial bleach (a.i. sodium hypochlorite) at a concentration of 50% for 2 min significantly reduced contamination (33.33%) and yielded higher percent survival (54.99%) compared to 20% and 10% concentrations. The addition of different levels of BAP to Murashige and Skoog (MS) medium resulted in shoot regeneration, with early shoot initiation (20 days) and the highest shoot length (9.89mm) observed at 3 mg L⁻¹ BAP after 4 weeks of incubation. The number of shoots was numerically higher at 5 mg L⁻¹, but this difference was not statistically significant. These results can be recommended for establishing dragon fruit explants *in vitro* at the surface sterilization and shoot initiation stages. Subsequent rooting and acclimatization stages, which were beyond the scope of this study, are still required before clean, off-season planting materials can be produced for large-scale production.

Keywords: *In vitro* dragon fruit; surface sterilization; BAP

1. Introduction

Dragon fruit production has become a lucrative industry in the Philippines. What used to be a backyard ornamental plant has now been propagated, cultivated, and fruit has been reaped, making what is now “The Philippines Dragon Fruit Industry” worth millions of pesos. Initiatives can be attributed to increased productivity in farms, since dragon fruit is recognized as a high-value crop [1]. The booming dragon fruit industry in the Philippines is hampered by problems with pests and diseases, inferior varieties, postharvest losses during transport, and the seasonality of dragon fruit, which results in limited supply of fresh fruit during the off season as well as of quality planting materials. To address these problems, science and technology (S&T) gaps must be identified to develop S&T interventions as solutions [1]. Traditionally, the propagation of *Hylocereus* spp. is carried out

through seed or vegetative cuttings [2]. However, propagation by cuttings is too slow to meet the demand for large-scale plantations due to a shortage of young nodes, and seeds are not genetically true-to-type, although the selected seedlings may resemble their parental plant in fruit quality [3]. Cross-pollination is also necessary for fruit setting. Hence, suitable planting materials were not available for large-scale cultivation of dragon fruit [4].

Plant tissue culture, or *in vitro* culture, is an alternative method for large-scale production of plantlets. Micropropagation of cacti facilitates the production of large numbers of plants from a relatively small stock [4]. Propagation of dragon fruit using tissue culture techniques will work well if the required conditions are met; these include selecting explants as planting material, sterilization, using suitable culture medium, maintaining aseptic conditions, and good temperature regulation. Once the explants were established on the initiation media, they were transferred to multiplication media for large-scale production [5]. Although conventional methods of propagation for these cacti are satisfactory, a micropropagation protocol has been developed to enable rapid release of improved clones or genotypes [3]. This study, therefore, was undertaken to regenerate dragon fruit *in vitro* to produce high-quality, off-season planting materials, facilitate disease-free exportation, and develop *in vitro* techniques, such as explant surface sterilization.

Globally, dragon fruit (pitahaya) has expanded from a minor specialty crop into an economically important fruit now cultivated across Asia, the Americas, and the Mediterranean. The rising demand for uniform, disease-free planting material has made *in vitro* propagation an increasingly relevant technology for tropical fruit crops. Reported micropropagation protocols, however, span several *Hylocereus* species and a wide range of cytokinin levels: Drew and Azimi-Tabrizi [3] induced bud development in *H. undatus*, Viñas et al. [6] tested 15, 30, and 60 μM BAP in *H. costaricensis*, and Shimomura and Fujihara [2] worked with *H. trigonus*. Because responses to cytokinins and to surface sterilants can differ substantially among *Hylocereus* species, comparisons across these studies must be interpreted with caution, and species-specific protocols remain limited for *H. polyrhizus*. Surface sterilization of cacti is itself challenging, as the soft, water-rich tissue is sensitive to hypochlorite, while thorns and areoles entrap air and hinder contact with the disinfectant. The 1, 3, and 5 mg L^{-1} BAP levels examined here (approximately 4, 13, and 22 μM) were selected to bracket the lower, more commonly effective end of the range used by Viñas et al. [6], consistent with the 1.0–3.0 mg L^{-1} optimum generally reported for BAP [7], while extending to 5 mg L^{-1} to test whether higher cytokinin would further improve shoot initiation. This study, therefore, focuses on *H. polyrhizus* and addresses two practical gaps for this species: identifying a commercial-bleach concentration that balances contamination control against tissue damage, and determining a BAP level suitable for shoot initiation.

2. Materials and Methods

2.1 Establishment of Mother Plants

Healthy stem cuttings (at least one year old) of dragon fruit were collected from Brgy. Maganhan, Baybay City, Leyte. The mother plants were potted in polybags containing a combination of sterilized carbonized rice hull, garden soil, and vermicompost (1:1:1) as the potting medium and placed inside a screenhouse near the laboratory. The cuttings were given proper care and nutrition while in the screenhouse. One week before explant collection, a fungicide (Dithane® M-45, active ingredient mancozeb 80% w/w, applied at approximately 7 g of product per liter of distilled water, equivalent to about 5.6 g mancozeb per liter) was sprayed to minimize contamination.

2.2 Surface Sterilization and Explant Preparation

Newly formed shoots of the dragon fruit cuttings ($\geq 10\text{cm}$ length) were collected from a one-month-old mother plant and served as explants (Fig. 1). The explants were washed thoroughly in running tap water for 30 minutes and in soapy water or liquid detergent solution for 1–2 minutes before rinsing. Explants were then surface-sterilized. In all treatments, the same sterilization procedure was used, but only the concentration of commercial bleach varied. First, the explants were soaked in a fungicide solution for 15 minutes, followed by a dip in 70% Ethanol. Next, explants were surface-sterilized using different concentrations of commercial bleach (50%, 20%, and 10%) for 2 minutes. Finally, the explants were soaked in

10% commercial bleach for another 2 minutes under a laminar flow hood and rinsed 3 times with double-sterilized distilled water before inoculation. The commercial bleach used in this study was [insert brand and manufacturer], which contained approximately 5.25% (w/v) sodium hypochlorite (NaOCl) as the labelled active ingredient; the 50%, 20%, and 10% treatments refer to volume/volume dilutions of this commercial product in distilled water, corresponding to approximately 2.6, 1.05, and 0.53% NaOCl, respectively.

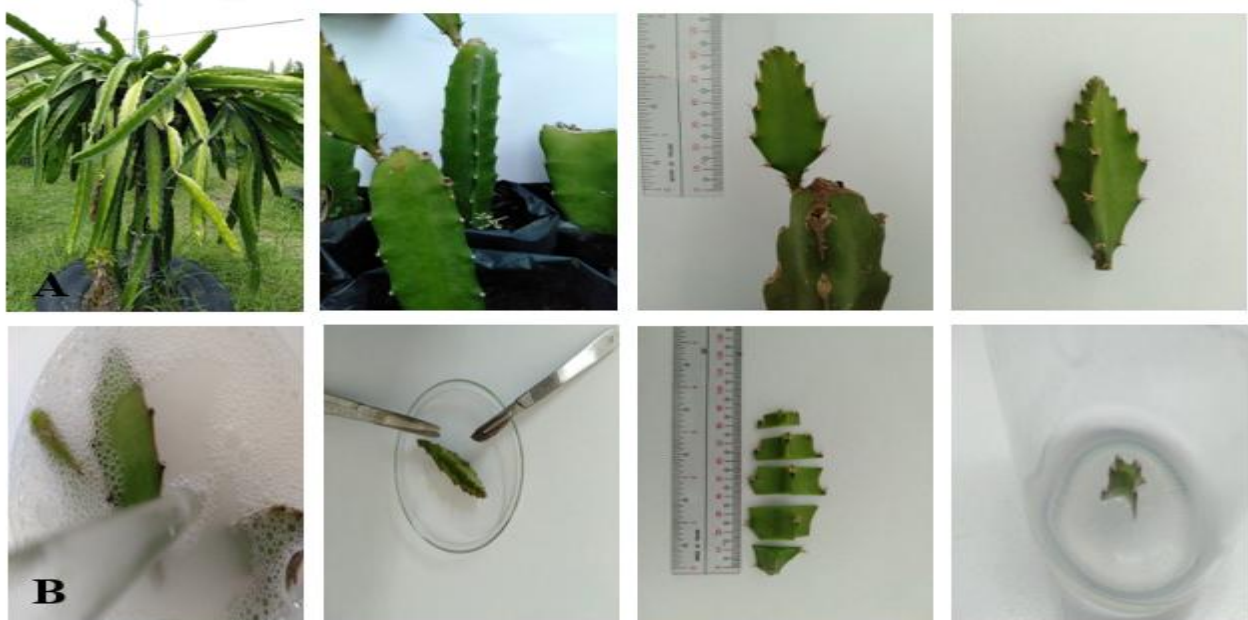


Figure 1. Source of explants (A) and preparation for surface sterilization (B) of red dragon fruit shoots for *in vitro* culture. (A1) one-month-old mother plant of red dragon fruit in the nursery; (A2) newly formed shoots (cladodes) borne on the potted mother plant; (A3) a harvested shoot cutting (≥ 10 cm) measured against a ruler; (A4) an isolated stem segment serving as the explant; (B1) explant washed in liquid detergent (soapy) solution; (B2) explant dipped in disinfectant solution using forceps; (B3) explant trimmed into segments and measured against a ruler; (B4) surface-sterilized explant rinsed in the bottom row, esterile distilled water before inoculation.

2.3 Culture Medium Preparation

In this experiment, stem segments with axillary buds were inoculated into Murashige and Skoog (MS, 1962) basal medium immediately after dissection. MS medium was supplemented with 3% sucrose (30 g L⁻¹) as a carbohydrate source and 0.5% agar as a gelling agent. The pH was adjusted to 5.8 with 1 N HCl or NaOH before dispensing into the culture vessels. Culture bottles containing the medium were covered with autoclavable plastic, autoclaved at 121°C for 20 minutes at 15 psi, and cooled before inoculation with explants.

2.4 Inoculation and Incubation

Sterilized explants were excised into stem segments with axillary buds at 1–1.5 cm in length, under the laminar flow hood using a sterilized scalpel and forceps. The damaged parts of the explants from the surface-sterilization procedure were trimmed before inoculating them into MS medium. The stem segments were planted upright into the prepared MS medium. Each culture bottle contains a single stem segment before being covered with plastic. The cultured bottles with the explants were incubated at $25 \pm 2^\circ\text{C}$ under white fluorescent light (light intensity of approximately $40 \mu\text{mol m}^{-2} \text{s}^{-1}$) in a 16h light and 8h dark photoperiod.

2.5 Shoot initiation of dragon fruit explants

To induce shooting, the clean explants were transferred to a MS medium formulation supplemented with different levels of 6-benzylaminopurine (BAP) at 1 mg L⁻¹, 3 mg L⁻¹, and 5 mg L⁻¹ as treatments. The medium was adjusted to pH 5.8 and dispensed at 20 mL per bottle before autoclaving at 15 psi for 20 minutes.

2.6 Experimental Design and Treatments

The study was laid out in a single-factor Completely Randomized Design (CRD). Treatments for surface sterilization used fifteen (15) stem segments of dragon fruit as samples, which were replicated four (4) times. For shoot initiation, treatments were replicated three (3) times, with ten (10) samples per replication. The smaller number of samples and replicates in the shoot initiation experiment reflects the limited number of clean, viable explants available after surface sterilization, as only explants that survived contamination and browning could be advanced to the shoot initiation stage. Treatments were designated as follows:

2.6.1 Surface Sterilization Experiment:

T1 – 70% Ethanol for 15 sec. + Fungicide for 15 mins. + 50% commercial bleach for 2 mins.

T2 – 70% Ethanol for 15 sec. + Fungicide for 15 mins. + 20% commercial bleach for 2 mins.

T3 – 70% Ethanol for 15 sec. + Fungicide for 15 mins. + 10% commercial bleach for 2 mins.

2.6.2 Shoot initiation Experiment:

T0 – MS basal medium alone

T1 – MS + 1 mg L⁻¹ BAP

T2 – MS + 3 mg L⁻¹ BAP

T3 – MS + 5 mg L⁻¹ BAP

2.7 Data Gathered

The parameters for surface sterilization include days to contamination from inoculation (dai), percent contamination (%), type of contaminants (e.g., fungi/bacteria), days to tissue browning (dai), percent tissue browning (%), degree of browning, and percent tissue survival (%). For shoot initiation, the data gathered include: days to shoot formation after transfer (days), percent shoot initiation (%), number of shoots per explant, and the weekly length of initiated shoots (mm).

2.8 Data Analysis

The collected data were analyzed using analysis of variance (ANOVA) in Statistix 10. Treatment means were compared using Tukey's Honest Significant Difference (HSD) at the 5% significance level.

3. Results and Discussion

3.1 Effect of commercial bleach as a surface sterilant for dragon fruit explants

Contamination, browning, and survival rate of dragon fruit stem segments after sterilization are presented in Table 1. The days to the first recorded contamination did not differ significantly among treatments. Appearance of contaminants was observed earlier in 20% and 10% commercial bleach, with an average of 4 days, whereas at 50% commercial bleach, appearance occurred on average 5 days after inoculation (dai). Highest contamination was reported in 10% commercial bleach at 71.67%, followed by 20% commercial bleach at 56.66%. Lowest contamination, on the other hand, was observed with 50% commercial bleach, with only 33.33% contamination. The study found that dragon fruit explants soaked in different levels of commercial bleach for 2 minutes indeed affected the percentage of contamination. Commercial bleach at 10% showed a higher contamination rate than 20% and 50% concentrations. The result suggested that the higher the level of commercial bleach used, the cleaner the cultures can be obtained. Similarly, in the study by Hesami et al. [8], increasing sodium hypochlorite concentration and immersion time reduced the percentage contamination of *Ficus religiosa*.

Table 1. Effect of concentration of commercial bleach on the contamination, browning, and survival of red dragon fruit explants *in vitro*

Treatments	Days to Contam (dai)	Percent Contam (%)	Type of contam	Percent Browning (%)	Degree of Browning	Percent Survival (%)
T1 - 70% Ethanol for 15 s + Fungicide for 15 mins + 50% commercial bleach for 2 mins	5	33.33 c	Fungi	11.65	3 a	54.99 a
T2 - 70% Ethanol for 15 s + Fungicide for 15 mins + 20% commercial bleach for 2 mins	4	56.66 b	Fungi	8.34	2 b	37.50 b
T3 - 70% Ethanol for 15 s + Fungicide for 15 mins + 10% commercial bleach for 2 mins	4	71.67 a	Fungi	6.67	1 b	25.00 c
CV (%)	9.76	9.89	-	30.58	25.20	15.22

Means with the same letter are not significantly different at 5% level (Tukey's HSD)

As shown in this study, sodium hypochlorite was very effective against bacterial contamination, since fungal contamination was the predominant contaminant among the cultures. Nakagawara et al. reported that even micromolar concentrations of sodium hypochlorite are sufficient to reduce bacterial populations [9] significantly. Contamination of cultures occurs despite explant sterilization, due to exogenously and endogenously present microbes. It should be noted that all three treatments shared the same 70% ethanol and fungicide steps and differed only in the concentration of commercial bleach, with no bleach-free control included. Consequently, the effects reported here are attributable specifically to the commercial bleach concentration rather than to the full sterilization protocol as a unit, and the absence of an untreated (ethanol- and fungicide-only) control is acknowledged as a limitation of the experimental design.

Using a higher concentration of commercial bleach in this study reduced contamination but induced browning of explants. Dragon fruit stem segments treated with commercial bleach showed the highest percent browning in 50% commercial bleach (11.65%), while the lowest was in 10% commercial bleach (6.67%). On the other hand, the degree of browning showed a significant difference between the commercial bleach used. Application of 50% commercial bleach showed a high degree of browning (>61% tissue browning), while the lowest degree of browning was recorded with 10% commercial bleach (1-20% tissue that turned brown). Commercial bleach at 20% showed a degree of browning comparable to 10% commercial bleach. The high concentration of commercial bleach (50%) increased the degree of browning, which damaged the soft tissues of dragon fruit stem segments (Fig. 2). Thus, the higher the percentage of commercial bleach used, the higher the percentage of aseptic culture, yet it also yields a greater number of explants that turn brown. Based on the results, regardless of the concentration of commercial bleach used in this experiment, explants showed browning due to bleach toxicity. Felek et al. [10] reported that surface sterilization with a higher concentration (0.5% w/v) of local bleach for 15 min or more. resulted in no contamination but a high rate (71.43%) of explant mortality of peach. Surface sterilization should not kill or inactivate the biological activity of explants, but should kill or inactivate contaminants. Explants must be surface-sterilized only by treatment with a disinfectant solution at suitable concentrations for a specified period [11]. Pitahaya (dragon fruit) tissue is very sensitive to disinfection with NaOCl or commercial bleach [6]. To reduce damage, it was necessary to initially treat larger explants with five to six areoles each before cutting them into smaller segments for *in vitro* culture.

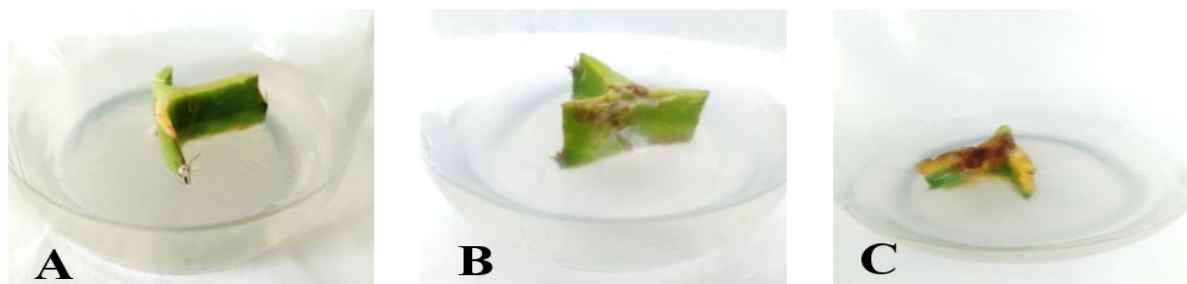


Figure 2. Degree of browning of red dragon fruit explants after surface sterilization using commercial bleach concentrations showing 1-20% tissue browning (A), 21-60% tissue browning (B), and >61% tissue browning (C)

3.2 Effect of sterilization on the survival of dragon fruit explants

The explant survival rate was relative to the contamination percentage, wherein high contamination resulted in low explant survival. Explants that turned brown also contributed to reduced culture survival. Hence, the survival of the explants was counted from samples that survived contamination and browning. A highly significant difference in percent survival was observed among treatments (Table 1). The survival rate of stem segments at 50% commercial bleach was over 54.99%, followed by 20% commercial bleach at 37.50%, and lowest at 10% commercial bleach at 25.00%. Commercial bleach has also been used to sterilize dragon fruit bud and stem segment explants to desired levels [6][4]. The survival rate of the two explants was over 60% in the first week of culture, but a higher percentage (85%) of survival was recorded in stem segments [4]. The higher survival rate in 50% commercial bleach compared to the other concentrations used in this study can be attributed to the effectiveness of sodium hypochlorite against all kinds of contaminants, which is the common active ingredient of the commercial bleach used in the study. However, compared with the study by Thinesh and Seran, only 54.99% survival was observed in this study. The difference could be attributed to the source of explants, the variety, and the culture medium used. Explant disinfection of cacti is difficult due to the softness of the tissue and the presence of thorns, which entrap air bubbles and prevent good contact with the disinfecting agent [6]. It is recommended that thorns cannot be removed from explants, otherwise axillary shoot growth does not occur [3]. It should be acknowledged that a survival rate of 54.99% is modest by tissue culture standards, as nearly half of the explants were lost even under the best treatment. This value is well below the 85% reported for stem segments by Thinesh and Seran [4] and therefore limits the immediate commercial viability of the protocol for large-scale production. The low survival reflects the competing demands of contamination control and tissue preservation in cactus explants, in which higher bleach concentrations reduce contamination but increase oxidative browning. Survival could likely be improved by adopting a sequential or stepwise sterilization regime, by incorporating antioxidants such as polyvinylpyrrolidone (PVP) or ascorbic acid into the rinses or culture medium to limit browning, and by pre-conditioning mother plants under controlled, low-contamination conditions before explant collection. These refinements are recommended before the protocol is scaled up for routine production. A comparable level of survival was reported for another cactus, in which medium sterilization of *Gymnocalycium mihanovichii* with 50 ppm chlorine dioxide gave the best survival of 54.17% [12], indicating that survival rates near 55% are not unusual for chemically sterilized cactus explants and that alternative sterilants such as chlorine dioxide may be worth evaluating for dragon fruit.

3.3 Shoot Initiation of Dragon Fruit Explants

The dragon fruit stem segments with axillary buds were cultured in MS medium supplemented with different levels of BAP to determine their in vitro response on shoot initiation and development. Variations in the response to shoot initiation of the explants revealed a significant difference in days to shoot initiation and percentage of shoot initiation as influenced by the different levels of BAP added into the culture medium (Table 2 and Fig. 3). No shoot initiation was observed in MS basal medium alone (T₀). Early shoot initiation was observed in medium containing 3 mg L⁻¹ BAP, which produced shoots at 20 days after

transfer (dat), followed by 1 mg L⁻¹ BAP and 5 mg L⁻¹ BAP, with comparable results at 38 and 34 days after transfer, respectively. Although the days to shoot initiation for 1 mg L⁻¹ (38 days) and 5 mg L⁻¹ (34 days) shared the same statistical grouping and were not significantly different, the four-day numerical difference may still be of practical relevance when the speed of culture establishment is important. In this study, at 20 days of initiation, 3 mg L⁻¹ BAP appeared to agree with the findings of Viñas et al. [6] and Drew and Azimi [3]. The shortest time required to initiate bud development of dragon fruit was 28 days after explant establishment in medium with 15, 30, or 60 µM BAP [6], while only 20 days were required for bud sprouting of *H. undatus* areoles with 5 µM N6 (2-isopentenyl) adenine, which extended to 26 d on hormone-free medium [3].

Table 2. Shoot initiation response of red dragon fruit explants as influenced by levels of BAP supplemented in MS medium *in vitro*

Treatment	Days to Shoot Initiation (day)	NO. of Shoots	Percent Shoot Initiation (%)
T0 - MS alone	-	-	-
T1 - MS + 1 mg L ⁻¹ BAP	38 a	1.00 ^{ns}	13.33 b
T2 - MS + 3 mg L ⁻¹ BAP	20 b	1.08 ^{ns}	30.00 ab
T3 - MS + 5 mg L ⁻¹ BAP	34 a	1.13 ^{ns}	46.67 a
CV (%)	11.39	9.95	24.85

Means with the same letter are not significantly different at 5% level (Tukey's HSD); ns = not significant

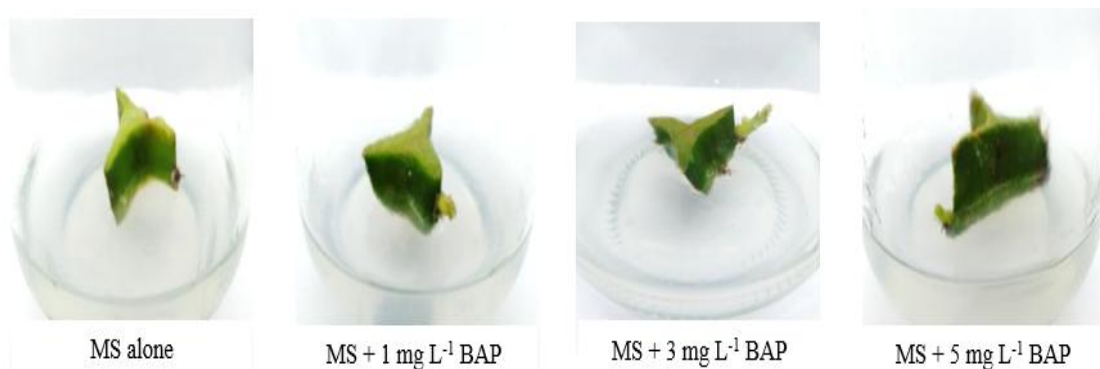


Figure 3. Formation of shoots from buds of stem segments of red dragon fruit explants *in vitro* as influenced by levels of BAP in MS medium

On the other hand, the delay in shoot initiation in treatment with higher BAP (5 mg L⁻¹) could be caused by the retardation of buds to produce shoots due to a high concentration of the hormone (BAP). This reduction in shoot formation may be due to BAP toxicity at higher concentrations. Narayanaswamy, as reported by Amer and Omar [13], stated that the toxicity caused by excess or prolonged exposure to growth regulators in the culture medium leads to genetic, physiological, and morphological changes, resulting in inhibition of proliferation. Past research indicates that there are optimal concentrations of BAP for specific plants. The study by Harahap et al. [14] appears to have limitations regarding the benefits of BAP at high doses on mangosteen explants. High concentrations of BAP caused treated explants to swell and produce many initial buds; however, those buds either did not grow into shoots or the shoots appeared stunted. The strong influence of cytokinin level on shoot response observed here is consistent with reports on other ornamental and cactus species. In a related cactus, *Gymnocalycium mihanovichii*, the highest shoot induction was obtained at low BA levels (0–2 mg L⁻¹) rather than at the highest level tested [16], in agreement with the present finding that 3 mg L⁻¹ BAP outperformed 5 mg L⁻¹ for shoot elongation. Besides the type and level of growth regulator, the strength and physical state of the basal medium also affect shoot multiplication, and full-strength MS medium has been reported to give the best shoot growth in other *in vitro* systems [15].

Culture medium supplementation with 1 mg L⁻¹ level of BAP is below the optimum level for shoot initiation, resulting in the lowest percentage of shoot formation. The explants cultured in 3 mg L⁻¹ BAP may have shown the earliest shoot initiation, but cultures in 5 mg L⁻¹ BAP had a higher percentage of initiated shoots (46.67%) and were significantly different from 1 mg L⁻¹ BAP (13.33%) in shoot initiation. Treatment with 3 mg L⁻¹ had 30% shoot initiation, which was statistically comparable to (i.e., not significantly different from) but numerically lower than that of 5 mg L⁻¹ BAP (46.67%). No significant difference in the number of shoots was observed across media with different BAP levels. The maximum number of shoots produced by an explant at 5 mg L⁻¹ was 3, with a collective average of 1.13 shoots. This was followed by 3 mg L⁻¹ and 1 mg L⁻¹, with average numbers of shoots of 1.00 and 1.08, respectively. All levels of BAP showed potential for promoting dragon fruit shoot growth *in vitro*. Aliyu and Mustapha reported that sprouting of cactus axillary bud requires low levels or no auxin, but always high levels of cytokinin [6]. Furthermore, they recommended that BAP be included for axillary bud sprouting in pitahaya cv Cebra. According to Machado et al. [17], explants transferred from a basic culture medium, diluted or not, and with reduced cytokinin concentration or cytokinin absence were enough to promote shoot growth. Cytokinins were known to stimulate shoot formation from explants *in vitro*, but the effect is more efficient depending on the amount applied to the medium and the explant's nutrient and hormone uptake rates. The useful BAP effects on the shoot's initiation are linked with the influence that this plant growth regulator has on the cellular division and shoot growth induction of the axillary buds inhibited by the plant apical dominance [18]. Arab et al. [19] also stated that cytokinin stimulates the initiation and activity of axillary meristems, which result in shoot induction and formation.

3.4 Growth of Dragon Fruit Shoots *in vitro* with Levels of BAP

Different levels of BAP influenced the length of the shoots of initiated dragon fruit shoots. No shoot growth was observed in cultures grown in MS alone, so the comparison was between cultures grown in medium supplemented with BAP. A significant difference in shoot length of dragon fruit explants was observed from the first to the fourth week of incubation among treatments with different levels of BAP (Table 3). The significant difference in shoot growth was consistent throughout the experiment, with cultures at 3 mg L⁻¹ obtaining the longest shoot length. After the 4th week of incubation or termination of the experiment, the highest length of shoots was observed in cultures grown in medium supplemented with 3 mg L⁻¹ BAP, with an average of 9.89 mm, which was significantly higher than cultures in 1 mg L⁻¹ BAP (3.67mm) and 5 mg L⁻¹ BAP (2.53mm). These showed a growth increment of 6.83mm in shoots at 3 mg L⁻¹ BAP compared to shoots at 1 mg L⁻¹ BAP and 5 mg L⁻¹ BAP, with only 1.84mm and 0.9 mm, respectively, at the fourth week. The higher shoot length of cultures in 3 mg L⁻¹ BAP could also be attributed to earlier shoot initiation in explants compared to other treatments, as shown in Table 3.

Table 3. Effect of levels of BAP in the length of shoots of red dragon fruit *in vitro* after four weeks incubation

Treatment	Weekly Length of Initiated Shoots (mm)			
	Week 1	Week 2	Week 3	Week 4
T0 - MS medium alone	-	-	-	-
T1 - MS + 1 mg L ⁻¹ BAP	1.83 b	1.83 b	2.67 b	3.67 b
T2 - MS + 3 mg L ⁻¹ BAP	3.06 a	4.39 a	7.17 a	9.89 a
T3 - MS + 5 mg L ⁻¹ BAP	1.63 b	1.77 b	2.03 b	2.53 b
CV (%)	14.12	16.80	18.69	27.53

Means with the same letter are not significantly different at 5% level (Tukey's HSD)

In general, BAP use in plants ranges from 1.0 to 3.0 mg L⁻¹. If the concentration is too high, it can cause shoot bud retardation, rendering the plant growth stunted, but the effect varies with different species [7] (pp. 175-205). In this experiment, retardation of shoot length was observed in 5 mg L⁻¹ BAP, while 1 mg L⁻¹ BAP was not optimal for shoot growth of the explants. Interestingly, increasing BAP concentration beyond the optimal level (3 mg L⁻¹) did not further improve shoot length but instead suppressed it in stem-segment explants. This reduction may be due to BAP's toxicity at higher concentrations. Narayanaswamy reported

that the toxicity caused by an excess of or prolonged exposure to growth regulators in the culture medium leads to genetic, physiological, and morphological changes and consequent inhibition of proliferation [13]. The dependence of plant growth on the type and concentration of growth regulator is well documented in other crops; for example, combined foliar application of gibberellic acid and salicylic acid markedly enhanced the growth and yield of kale [20], and immersion in indole-3-butyric acid promoted bud germination and growth of *Caladium* tuber sections [21], reinforcing that an appropriate, rather than maximal, level of growth regulator is required for optimal growth.

4. Conclusions

A 50% commercial bleach concentration for 2 mins produced the lowest percent contamination and the highest percent survival of red dragon fruit explants. However, it showed the highest percentage of browning to explants due to commercial bleach toxicity. Among the BAP levels tested, 3 mg L⁻¹ supplemented to MS medium is recommended for shoot initiation of *H. polyrhizus* because it gave the earliest shoot initiation (20 days) and the greatest shoot length (9.89 mm), and produced a percent shoot initiation (30%) that was statistically comparable to that at 5 mg L⁻¹ (46.67%). Although 5 mg L⁻¹ gave the highest number of shoots per explant, this difference was not statistically significant, and the same concentration retarded shoot length; it is therefore not recommended as the optimum. Taken together across all performance parameters, 3 mg L⁻¹ BAP represents the best overall treatment for shoot initiation in this study. In this experiment, retardation of shoot length was observed in 5 mg L⁻¹ BAP, while 1 mg L⁻¹ BAP was not optimal for shoot growth of the explants. Interestingly, increasing BAP concentration beyond the optimal level did not further improve any parameter associated with shoot initiation and instead suppressed shoot length in stem-segment explants. It should be noted that this study covered only the surface sterilization and shoot initiation stages; rooting and acclimatization were not evaluated. Further work on rooting, elongation, and ex vitro acclimatization, together with strategies to improve explant survival (e.g., sequential sterilization, antioxidant supplementation, and mother-plant pre-conditioning), is therefore required before a complete micropropagation protocol for large-scale production of *H. polyrhizus* planting materials can be established.

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