Journal of Tropical Crop Science Vol. 12 No. 3, October 2025
www.j-tropical-crops.com

Received 01/08/2025; Revised 20/10/2025; Accepted 24/10/2025
https://doi.org/10.29244/jtcs.12.03.663-671

Optimizing Tissue Culture for Yellow Dragon Fruit (*Selenicereus megalanthus*) Propagation: Enhancing Shoot and Root Induction

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Abstract

Yellow dragon fruit (*Selenicereus megalanthus*) "Palora" variety is a tropical cactus recognized for its yellow pericarp, white mesocarp, and black seeds. Dragon fruits are also known as pitaya. The yellow pitaya variant is regarded as the sweetest and most nutrient-dense, with high commercial value among the commonly cultivated pitaya types. However, large-scale cultivation of yellow dragon fruit remains limited by propagation constraints. The conventional propagation method using vegetative stem cuttings is labour-intensive and results in inconsistent growth and genetic variability. Tissue culture has emerged as a viable biotechnological alternative, enabling rapid multiplication of genetically uniform and disease-free plantlets under controlled conditions. The study aimed to optimize tissue culture medium for enhancing shoot induction on yellow dragon fruit. The experiments were conducted in two steps. The first step evaluated the explant response in MS medium containing 3 mg.L^{-1} BAP and varying NAA concentrations (0.1, 0.2, 1, 2, and 3 mg.L^{-1}). The results showed that an elevation in NAA concentration promoted progressive growth in shoots, roots, and callus. However, when NAA was further augmented to 3 mg.L^{-1}, shoot and root formation were repressed, while callus formation increased. The second step evaluated the combined effects of auxin and cytokinin on shoot formation. A factorial treatment design was applied using combinations of three NAA concentrations (1, 2, and 3 mg.L^{-1}) and three BAP concentrations (1, 2, and 3 mg.L^{-1}), resulting in nine treatment combinations, with the lowest concentration (NAA 1 mg.L^{-1} + BAP 1 mg.L^{-1}) as the control. Statistical analysis revealed that NAA, BAP, and their interaction had a significant effect on the number of shoots. The optimal treatment for producing the highest number of shoots is a combination of NAA at 3 mg.L^{-1} and BAP at 2 mg.L^{-1}.

Keywords: BAP, callus, Cactaceae, cytokinin, NAA

Introduction

Yellow dragon fruit (*Selenicereus megalanthus*) "Palora" variety is regarded as the sweetest and most nutrient-dense among other commonly grown pitaya types. Its nutritional profile and therapeutic benefits include vitamins, minerals, fibre, and antioxidants, particularly betalains and polyphenols, with minimal fat content (Biswas et al., 2024). The properties of pitaya fruits exhibit its wide array of health-promoting benefits. Reported advantages are antioxidative support, enhanced digestion, regulation of blood glucose levels, bone strengthening, reduction of cardiovascular risks, enhanced hydration, and improved iron absorption (Nishikito et al., 2023; Biswas et al., 2024). Despite its commercial value, propagation limitations restrict the large-scale cultivation of yellow dragon fruit. The conventional method of vegetative stem cuttings is employed in the labor-intensive, genetically variable, and inconsistent propagation method (Rehman et al., 2023).

Another challenge in the conventional cutting propagation of pitaya is the susceptibility of cuttings to environmental changes and pathogenic infections. Due to its vulnerability, production costs remain high, resulting in market prices ranging from IDR 500,000 to IDR 900,000 per kg. On the other hand, the price of white dragon fruit ranges from IDR 80,000 to 100,000, while the purple one is IDR 20,000 to IDR 30,000 per kg, respectively. These differences reduce market accessibility and highlight the need for improved propagation strategies.

Tissue culture has emerged as a viable biotechnological alternative, enabling rapid

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multiplication of genetically uniform and disease-free plantlets under controlled conditions (Rehman et al., 2023). Faster rates of regeneration, greater consistency in plant quality, reduced space requirements, and independence from external environmental factors are just a few advantages of this strategy (Aremu et al., 2020). Due to these qualities, it can be utilized on a commercial scale. These attributes make it suitable for commercial-scale applications. Unfortunately, the effective and efficient protocol for tissue culture of *S. megalanthus* remains limited, particularly in terms of shoot induction. This phase is critical in micropropagation, as it initiates organ development and facilitates efficient subculturing (Rehman et al., 2023). Thus far, the published methods often result in excessive callus formation, which impedes direct shoot organogenesis and compromises propagation efficiency.

Callus refers to an undifferentiated plant cell that forms in response to wounding and high levels of auxin. It becomes problematic when it expands uncontrollably, delaying the formation of organized shoots or roots (Ikeuchi et al., 2013). In such cases, the tissue remains undifferentiated for extended periods (non-organogenic), prolonging the regeneration process and may result in somaclonal variation, leading to inconsistent plantlet traits (Shin et al., 2020). Morphologically, non-organogenic callus often appears friable, translucent, or spongy (Aremu et al., 2020). Excessive callus formation will compete for nutrients and space, hindering organ formation and development, and ultimately reducing the overall efficiency of plant tissue culture. Therefore, the optimum level of auxin and cytokinin is determined to induce a competent callus capable of shoot or root formation. Reducing callus production is essential to ensure uniformity and maintain the genetic fidelity of plantlets, particularly in large-scale production systems.

Studies on related cactus species suggest that the success of tissue culture protocols is highly dependent on the interaction among plant growth regulators. Combinations of cytokinins and auxins, such as benzylaminopurine (BAP) and naphthaleneacetic acid (NAA), have shown varying results (Rehman et al., 2023). The following protocols have been tested: half-strength Murashige and Skoog (MS) medium with 2 $mg.L^{-1}$ BAP and 0.5 $mg.L^{-1}$ NAA; 2 $mg.L^{-1}$ BAP with 0.2 $mg.L^{-1}$ NAA; and 2 $mg.L^{-1}$ BAP with 0.5 $mg.L^{-1}$ NAA (Nishikito et al., 2023). Unfortunately, their effectiveness has been limited by adverse results, including persistent callus development, delayed shoot formation, and root overgrowth (Biswas et al., 2024).

Advances in plant biotechnology, sustainable agriculture, and global food security are all aided by improved tissue culture protocols (Aremu et al., 2020). The current study focuses on optimizing full-strength MS medium supplemented with BAP and NAA, hypothesizing that a 2 $mg.L^{-1}$ BAP and 3 $mg.L^{-1}$ NAA combination is optimal for shoot induction in *S. megalanthus* (Rehman et al., 2023). By modifying this protocol, we would like to establish a reproducible method that maximizes shoot regeneration while minimizing undesired callus proliferation. The broader implications of this work extend beyond a single species. This research supports the development of effective and scalable propagation systems for valuable horticultural crops.

Materials and Methods

Plant Materials and Sterilization

This study was conducted in Dirga Laboratory, Ciangsana, Bogor, a sterile plant tissue culture facility operating under controlled environmental conditions to ensure aseptic handling and prevent microbial contamination. Initial explants were taken from axillary shoots of yellow dragon fruit grown under the greenhouse. The initial sterilization of explants was conducted outside the laminar flow cabinet to remove dirt, residues, and microorganisms (Infante, 1992). Explants were washed in 100 mL of distilled water containing 5 mL of dish soap, shaken vigorously for 5 minutes (Román et al., 2014). Then, they were rinsed thoroughly with fresh distilled water to remove all soap residues. It is a crucial step to prevent interference with subsequent sterilization (Rehman et al., 2023). To remove fungal and bacterial contaminants, explants were soaked in a solution of dithane M-45 (mancozeb 80%) and agrept 20WP (streptomycin sulphate 20%), each at 2 $g.L^{-1}$, for 1 hour (Lee and Chang, 2022). This antimicrobial treatment is crucial for reducing contamination during in vitro culture.

The second sterilization phase was performed inside a laminar flow cabinet under sterile conditions. To remove residual chemicals, the explants were rinsed for three minutes per rinse in sterile distilled water, with gentle agitation to remove residual chemicals (Aremu et al., 2020). Explants sterilization was continued by dipping them in 70% ethanol for 30 seconds. This step aimed to denature microbial proteins with minimum tissue damage due to short exposure time (Alsanie, 2025). Subsequently, explants were rinsed with sterile water for 3 minutes to remove residual ethanol. The explants were further disinfected using a 20% bleach (sodium hypochlorite) mixed with Tween 20 (three drops per 100 mL). Tween 20 reduced

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surface tension, allowing bleach to access hidden crevices (Trivellini et al., 2020). Explants were soaked in this solution for 10 minutes with constant agitation. The final steps of the sterilization procedure involve removing traces of bleach by rinsing the explants in distilled, sterile water three times, each rinse lasting 3 minutes (Mahmod et al., 2021). Excess moisture was blotted using sterile tissues to prepare the samples for transfer to the culture media.

Experimental Design and Growth Measurements

This study employed a completely randomized design, consisting of two experiments. The first experiment tested different concentrations of NAA (0.1, 0.2, 1, 2, 3 mg.L^{-1}) in MS medium containing BAP 3 mg.L^{-1}. It used ten replications with 14-15 explants per replication. The growth measurements were conducted by counting the number of explants that formed callus, shoots, and roots at 6 weeks after planting. The second experiment tested two factors: auxin (NAA 1, 2, 3 mg.L^{-1}) and cytokinin (BAP 1, 2, 3 mg.L^{-1}) concentrations, with NAA 1 mg.L^{-1} + BAP 1 mg.L^{-1} as the control treatment. Each treatment was replicated five times, producing 45 experimental units. Each experimental unit consists of 4-6 explant samples, so a total of around 180 explants were used in this experiment. The observation and measurements were performed on the number of shoots and roots produced from the explant at 12 weeks after planting.

To estimate overall shoot production efficiency, the total shoot number prediction was calculated using the formula:

Total shoot number = (shoot formation percentage × average shoot number per explant × 50).

The increase of regeneration was calculated by comparing the control (NAA 1 mg.L^{-1} + BAP 1 mg.L^{-1}) with the formula:
Increase in shoot production = (shoot number on combination treatment – shoot number on control)/ shoot number in control) X 100%

This formula provided a standardized measure of multiplication potential for each treatment, enabling comparison of hormonal effectiveness across NAA and BAP combinations.

Preparation of Culture Media

The preparation of Murashige and Skoog (MS) basal medium requires accuracy to ensure reproducibility and optimal tissue development in vitro. The steps of media preparation are preparation of MS stock solutions, media supplementation, pH adjustment, solidification, and sterilization (Murashige and Skoog, 1962; Lee and Chang, 2022). The MS medium was prepared by combining specific volumes of concentrated stock solutions into a 1 L beaker, following standard protocols commonly used in plant tissue culture (Bello-Bello et al., 2021).

The detail component of MS medium are ammonium Nitrate (NH_4NO_3) that serves as a primary nitrogen source in the MS medium (Mahmod et al., 2021), potassium nitrate (KNO_3) that provides additional nitrate ions to balance nitrogen uptake (Hua et al., 2014), micronutrient Stock A (potassium phosphate (KH_2PO_4), boric acid (H_3BO_3), potassium iodide (KI), sodium molybdate ($Na_2MoO_4.2H_2O$), and cobalt (II) chloride hexahydrate ($CoCl_2.6H_2O$). Calcium chloride dihydrate ($CaCl_2.2H_{2O}$) serving as a calcium and chloride source for cell wall stabilization and signaling (Román et al., 2014), micronutrient Stock B contained magnesium sulfate heptahydrate ($MgSO_4.7H_2O$), manganese sulphate monohydrate ($MnSO_4.H_2O$), zinc sulphate heptahydrate ($ZnSO_4.7H_2O$), and copper (II) sulphate pentahydrate ($CuSO_4.5H_2O$) (Trivellini et al., 2020), iron-EDTA stock (A chelated iron complex of ethylenediaminetetraacetic acid disodium salt dihydrate ($Na_2EDTA.2H_2O$) and iron (II) sulphate heptahydrate ($FeSO_4.7H_2O$)) (Rehman et al., 2023), myo-Inositol that function as a growth stimulant and membrane stabilizer (Aremu et al., 2020), and vitamine stock containing thiamine, niacin, pyridoxine, and glycine, function to support cellular metabolism and enzyme function (Alsanie, 2025). The other crucial component in culture media is plant growth regulators (NAA and BAP) that will induce plant cell multiplication and differentiation. Sucrose is added to provide a carbon source essential for energy and metabolic functions of the plant cell (Hua et al., 2014). Finally, distilled water was added to bring the total volume of the solution to 1 L.

To ensure nutrient availability for the explants, the pH of the medium was adjusted to 5.8 using sodium hydroxide (NaOH) and hydrochloric acid (HCl), as measured by a pH meter (Bello-Bello et al., 2021). The optimum pH supports the optimal nutrient uptake and enzyme activity (Mahmod et al., 2021). To solidify the medium, 0.8% agar was added and dissolved into the medium, then heated to boiling, which ensures uniform solidification and appropriate texture for explant placement (Mustafa and Saad, 2020). Subsequently, the medium was poured into sterile culture bottle (25 mL per culture bottle), then sterilized to eliminate microbial contaminants by using autoclave that was set for 121°C and a pressure of 120.66 kPa (15 psi) for 30 minutes, a standard sterilization protocol for plant tissue culture media (Murashige and Skoog, 1962; Trivellini et al., 2020). After autocloaving, the media were allowed to cool,

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enabling the medium to solidify into a gel suitable for in vitro growth (Pelah et al., 2002).

Explants Preparation, Inoculation, and Culture Conditions

Explants were taken from the in vitro shoot culture stock of yellow dragon fruit. The procedures are presented in Figure 1. In vitro shoot tips were cut from the mother shoots, approximately 1-2 cm segments for uniformity (Bello-Bello et al., 2021). Under aseptic conditions, they were transferred to the treatment medium as explained in the experimental design section. Tools used for cutting and transfer were sterilized, and procedures were carried out inside the laminar cabinet to avoid contamination. The explants were maintained in a culture room with a temperature range of 25±2°C. The photoperiod was set at 16 hours of light and 8 hours of darkness.

Data Analysis

The number of explants forming callus, shoots, and roots from Experiment 1 was calculated to determine the percentage of explant responses at different NAA concentrations. For Experiment 2, we conducted an Analysis of Variance (ANOVA) for the shoots and roots to identify statistically significant differences in shoot proliferation across the treatments with varying combinations of plant growth regulators. This line of study falls under quantitative data analysis in plant tissue culture, focusing on the statistical evaluation of hormonal effects on morphogenetic responses.

Results

Experiment 1. Explant Responses to Elevated NAA Concentrations

This experiment aimed to determine the optimum level of NAA for maximizing shoot formation of *S. megalanthus* explants. The study evaluated the regenerative potential of the explants in MS medium supplemented with different NAA concentrations (0.1, 0.2, 1, 2, 3 mg.L^{-1}) and a similar concentration of BAP (2 mg.L^{-1}). Figure 2 shows the effect of elevating NAA concentration on callus induction, root formation, and shoot regeneration. The results indicate that increased NAA concentration endorses overall tissue responses, including callus, root, and shoot development. Root formation displayed the highest percentage across all treatments, followed by shoot and callus formation. The optimal concentration for root and shoot induction was found to be 2 mg.L^{-1} NAA. Beyond NAA 3 mg.L^{-1}, both responses declined. In contrast, callus formation exhibited a linear trend, indicating that the explants continued to produce callus even at the highest NAA concentration tested (3 mg.L^{-1}). These findings recommend that NAA 2

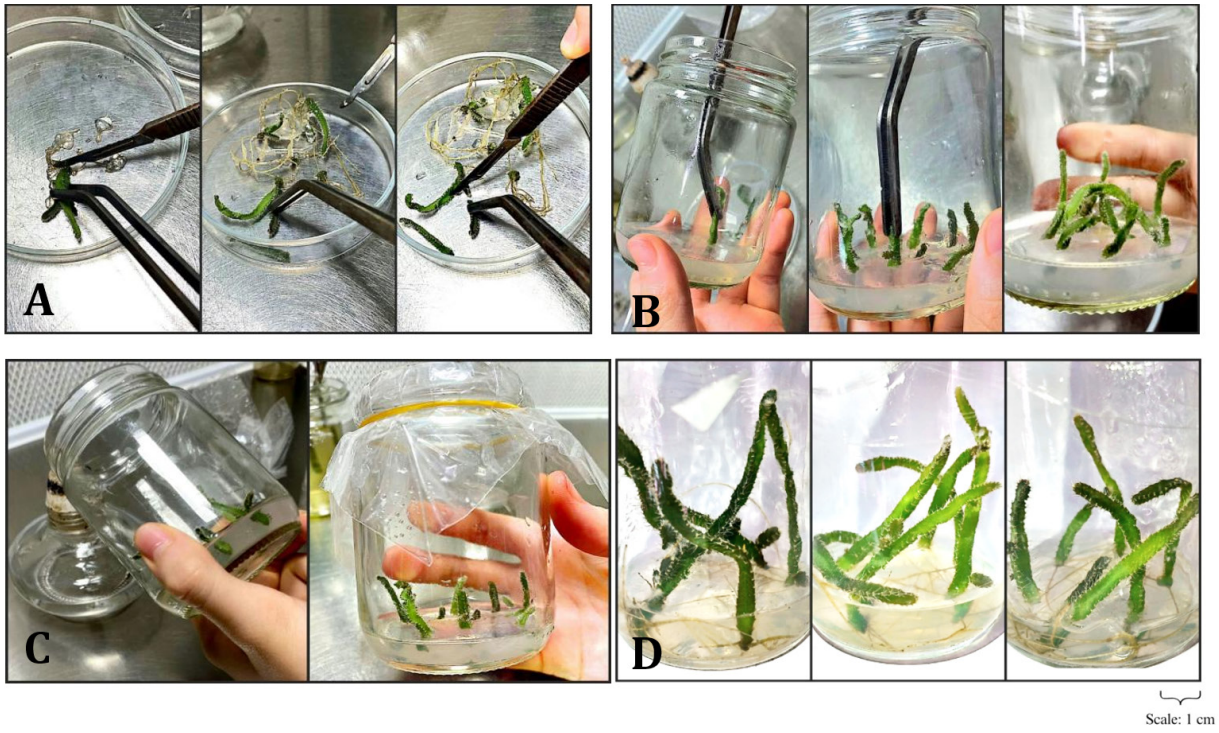

Figure 1. The general steps of yellow dragon fruit tissue culture: A. Initial shoots trimming on the petri dish, B. Shoots were planted in the new bottle medium, C. Sterilizing and sealing the jar, D. Culture incubation in the tissue culture room for growth evaluation.

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mg.L^{-1} supports balanced organogenesis; however, NAA 3 mg.L^{-1} may still be favourable for maximizing shoot production.

Experiment 2. Shoot Induction on Different NAA and BAP Concentrations

This experiment intends to identify the optimal concentration of NAA and BAP to produce the maximum number of shoots on *S. megalanthus* explants. The results of this study highlight the regenerative capacity of *S. megalanthus* explants in MS medium supplemented with varying concentrations of BAP and NAA. Shoot regeneration was analysed over 12 weeks after planting. The findings demonstrate the efficacy of these plant growth regulators (PGRs) in stimulating shoot formation. The results show that two factors, treatments (NAA and BAP) and their interaction, significantly affect the number of shoots (Table 1).

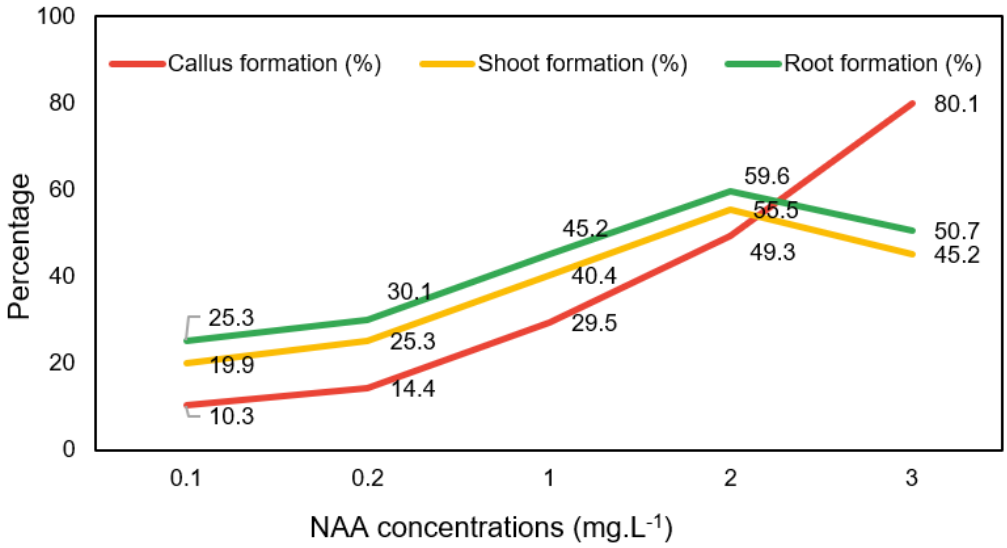

Figure 2. Proportion of callus, shoots, and root formation at varying NAA concentrations (0.1–3.0 mg.L^{-1}) at 6 weeks after planting. The graph illustrates that callus formation increases linearly with NAA concentration, while shoot and root formation exhibit optimal development at intermediate concentrations. Excessive NAA concentrations (3.0 mg.L^{-1}) reduced shoot and root formation.

Table 1. Average shoot number per explant of *S. megalanthus* under varying concentrations of NAA and BAP at 12 weeks after planting

NAA (mg.L^{-1})	BAP (mg.L^{-1})		
	1	2	3
	Shoot number per explant		
1	2.0Cc	3.0Cb	3.3Ba
2	3.6Bb	4.1Ba	4.2Aa
3	4.5Ab	4.8Aa	4.2Ac
	Total shoot number prediction		
1	53	78	76
2	139	170	187
3	198	226	122
	Increases in shoot production compared to the control (%)		
1	-	47.2	43.4
2	162.3	22.7	252.8
3	273.5	326.4	130.2

Notes: Values followed by a similar capital letter in the same column, or similar small letter in the same row, are not significantly different according to the DMRT at α=0.05.

Received 01/08/2025; Revised 20/10/2025; Accepted 24/10/2025
https://doi.org/10.29244/jtcs.12.03.663-671

Table 1 displays the average number of shoots produced per explant in each NAA and BAP concentration combination. It shows NAA 3 mg.L^{-1} and BAP 2 mg.L^{-1} as the optimum combination that yielded the highest shoot number, averaging 4.8 shoots per explant. A predictive estimate, calculated by multiplying the percentage of shoot formation by the average number of shoots for a batch of 50 explants, underpins this treatment as the most effective. Specifically, the combination of NAA 3 mg.L^{-1} and BAP 2 mg.L^{-1} leads to a projected shoot production of 226 shoots per 50 explants, corresponding to 326% increase in regenerative performance relative to the control treatment (NAA 1 mg.L^{-1} + BAP 1 mg.L^{-1}). This suggests a higher regenerative performance and optimal hormonal interaction for shoot induction.

Figure 3 illustrates the percentage of shoot formation in *S. megalanthus* explants under varying combinations of NAA (1, 2, 3 mg.L^{-1}) and BAP (1, 2, 3 mg.L^{-1}). At NAA 1 mg.L^{-1} with BAP 1, 2, or 3 mg.L^{-1}, the percentage of shoot formation is approximately 50%, which is comparable to the control treatment (NAA 1 mg.L^{-1} +BAP 1 mg.L^{-1}). Increasing NAA to 2 mg.L^{-1} noticeably promotes shoot formation, reaching 77%, 83%, and 89% at BAP 1, 2, and 3 mg.L^{-1}, respectively.

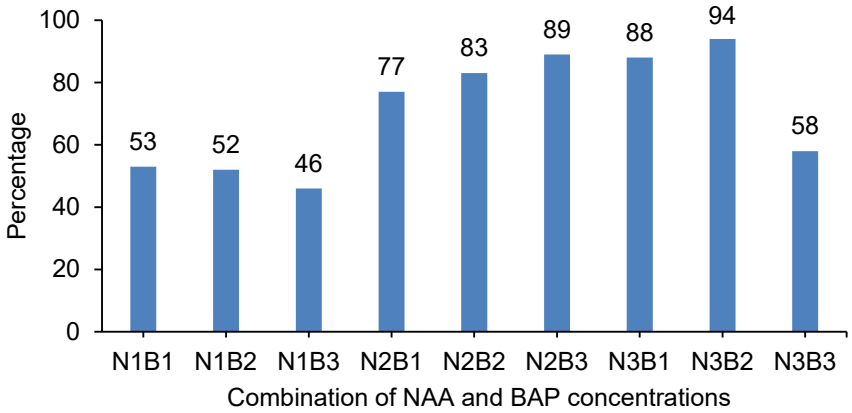

Figure 3. Percentage of shoot formation of *S. megalanthus* explants varying the concentration of NAA and BAP at 12 weeks after planting. N1, N2, N3 are NAA 1, 2, 3 mg.L^{-1}, B1, B2, B3 are BAP 1, 2, 3 mg.L^{-1}.

Figure 4. Representative of explants performance grown in a combination of NAA 3 mg.L^{-1} and BAP 2 mg.L^{-1} over 12 weeks, highlighting 3-10 (average 4.8 shoots) were observed in each explant.

Received 01/08/2025; Revised 20/10/2025; Accepted 24/10/2025
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These values represent relative increases of 27%, 33%, and 39% compared to the control treatment (NAA 1 mg.L^{-1} + BAP 1 mg.L^{-1}). At NAA 3 mg.L^{-1}, shoot formation remained high at 88% and 98% for BAP 1 and 2 mg.L^{-1}, corresponding to improvements of 38% and 48% relative to control treatment (NAA 1 mg.L^{-1} + BAP 1 mg.L^{-1}). However, at BAP 3 mg.L^{-1}, shoot formation declined to 58%, representing only an 8% increase compared to the control treatment. These results suggest that NAA concentrations between 2 and 3 mg.L^{-1} are optimal for promoting shoot formation, particularly in combination with BAP at 1-2 mg.L^{-1}.

Discussion

The recent research highlights the importance of selecting effective plant growth regulators (PGRs) and supplements for thriving tissue culture in *S. megalanthus*. Experiment 1 demonstrates that varying NAA concentrations, while maintaining a fixed BAP concentration in the medium, elicit specific responses. With BAP at 3 mg.L^{-1} in the medium, increasing NAA to 3 mg.L^{-1} will linearly promote callus growth. In contrast, root and shoot induction will reach their maximum induction at NAA 2 mg.L^{-1}, then decrease at NAA 3 mg.L^{-1}. The ratio of auxin and cytokinin plays a critical role in determining the fate of the explants. A high ratio of auxin to cytokinin promotes suitable root formation, a high cytokinin to auxin ratio promotes shoot formation, and the balance of these two hormones tends to promote callus growth (Ikeuchi et al., 2013).

BAP plays a key role in promoting shoot proliferation, as seen in previous studies on pitaya regeneration (Dahanayake and Ranawake, 2012; Hua et al., 2014). BAP has a crucial role in mitotic activity in the apical meristem, specifically in the shoot apical meristem, and then promotes lateral bud formation by suppressing apical dominance. It also supports chlorophyll synthesis and vacuole expansion for shoot elongation (Gonzales-Alvarado and Cardoso, 2024). On the other hand, NAA's central roles are to enhance cell enlargement, vascular differentiation, and facilitate early root formation (Rehman et al., 2023). They maintain a hormonal balance that favours direct organogenesis over unstructured callus growth (Román et al., 2014; Hua et al., 2014). While BAP and NAA are effective, other PGRs require careful handling. For example, TDZ, though potent, can cause hyperhydricity at high concentrations (Pelah et al., 2002). 2,4-D is linked to somaclonal variation and reduced genetic stability in regenerants (Dahanayake and Ranawake, 2012).

Excessive hormone concentration may harm the explants. For instance, in Experiment 2 of this research, explants exposed to 3.0 mg.L^{-1} BAP exhibited reduced shoot initiation and stunted growth, signs of hyperhydricity —a physiological disorder common under high cytokinin concentrations. The similar symptom, also reported by Aremu et al. (2020) and Biswas et al. (2024), implies that an excessive BAP concentration in the culture medium may disrupt cellular differentiation and elongation, leading to diminished shoot quality. Comparatively, control explants grown in MS media without PGRs exhibited minimal shoot initiation, reinforcing the necessity of BAP and NAA for shoot induction in *S. megalanthus* (Rahman, 2015). The balance between BAP and NAA also influenced the overall morphology of regenerated shoots. Explants cultured in media with an appropriate auxin-to-cytokinin ratio exhibited uniform shoot development, characterized by well-defined nodes and internodes. Conversely, excessive BAP concentrations developed shoots with abnormal clustering, reduced leaf expansion, and vitrification symptoms, further emphasizing the importance of optimizing PGR levels. Previous studies on plants suggested that an imbalance in growth regulators can negatively impact organogenesis by altering cellular division patterns and metabolic activity (Nunez et al., 2014; Lee and Chang, 2022).

In the present study, the combination of 3 mg.L^{-1} NAA and 2 mg.L^{-1} BAP produced an average of 4.8 shoots per explant, equivalent to a multiplication rate of approximately 226 shoots per 50 explants, marking a 326.4% increase compared to the control treatment. This result aligns closely with findings by Bello-Bello et al. (2021), who reported a multiplication coefficient of 4.6 shoots per explant in *Hylocereus undatus* using temporary immersion bioreactors supplemented with 2 mg.L^{-1} BAP and 0.5 mg.L^{-1} NAA. However, while Bello-Bello's system employed liquid-phase immersion to enhance aeration and nutrient uptake, the current study achieved comparable multiplication rates under solid MS medium conditions, suggesting that optimization of hormonal balance alone can yield similar efficiency without specialized bioreactor systems. The slight variation in results may be attributed to species-specific hormonal sensitivity, as *S. megalanthus* often exhibits slower in vitro responsiveness compared to *H. undatus*. Nonetheless, both studies confirm that a moderate BAP concentration (2–3 mg.L^{-1}) in combination with a supportive auxin level (2–3 mg.L^{-1} NAA) is optimal for shoot induction and proliferation in cacti.

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Conclusions

The results indicate that the optimal concentration of plant growth regulators for *S. megalanthus* shoot induction is 2.0 mg.L^{-1} BAP combined with 3 mg.L^{-1} NAA. Under these conditions, explants demonstrated the highest rates of shoot regeneration. The optimum concentrations for root induction are BAP at 3 mg.L^{-1} and NAA at 2 mg.L^{-1}, which yield the highest percentage of root formation in the explants. These findings provide tissue culture protocols for *S. megalanthus*. However, further refinement of PGR concentrations is still necessary to ensure optimal performance and a high success rate of the best plantlets at the acclimatization stage.

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Received 01/08/2025; Revised 20/10/2025; Accepted 24/10/2025
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