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RESEARCH ARTICLE

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Basal Media–BAP Interactions Regulate In Vitro Shoot Induction in Anthurium plowmanii

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Abstract

Anthurium plowmanii is an economically valuable ornamental foliage plant, yet its commercial production is constrained by slow natural propagation. This study investigated the effects of three basal media—full-strength Murashige and Skoog (MS), half-strength MS (½ MS), and Hyponex—and four concentrations of 6-Benzylaminopurine (BAP) (0.00, 2.22, 6.66, and 13.32 µM) on in vitro shoot induction and multiplication. A two-factor factorial experiment in a completely randomized design was used, and responses were measured as days to shoot initiation, shoot number, shoot height, and leaf number. Results showed that both basal medium and BAP significantly affected all parameters, with significant interaction effects for shoot number and leaf number. The combination of ½ MS and 13.32 µM BAP produced the highest number of shoots (10.7) and leaves (4.8) per explant, while high BAP levels accelerated shoot initiation but reduced shoot height. Hyponex medium produced the lowest regeneration responses across all BAP levels. These results highlight the synergistic influence of moderate nutrient strength and high cytokinin on shoot organogenesis and provide a practical protocol for rapid and uniform propagation of *A. plowmanii*.

KEYWORDS
Anthurium plowmanii; in vitro propagation; Murashige and Skoog; Hyponex; 6-Benzylaminopurine; basal medium; shoot multiplication.

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1 | INTRODUCTION

Anthurium plowmanii, commonly known as the *Wave of Love* anthurium, is an increasingly popular ornamental foliage plant prized for its large corrugated leaves, glossy texture, and distinctive wavy margins. Native to South America, this species has gained significant commercial importance in the global floriculture industry as a high-value foliage houseplant and landscaping material (Chakraborty et al., 2023). In Indonesia and other tropical regions, *A. plowmanii* is sought after by nurseries and collectors, driving a rapid rise in market demand (Putri et al., 2024). However, large-scale production of this species is hindered by its slow natural propagation through seeds or vegetative offshoots, which limits the availability of uniform, disease-free planting material (Rohini et al., 2022). Seeds are often scarce, exhibit erratic germination, and produce highly heterozygous progeny, while vegetative propagation is constrained by low multiplication rates and the risk of transmitting systemic pathogens (Gómez et al., 2023). These constraints necessitate the development of efficient clonal propagation systems to meet the growing demand for high-quality *A. plowmanii* plants.

In vitro micropropagation offers an effective solution to overcome the limitations of conventional propagation. Tissue culture enables rapid multiplication of genetically uniform plantlets within a controlled, pathogen-free environment, allowing year-round production independent of seasonal constraints (Mhatre et al., 2023). For aroids like anthuriums, micropropagation also facilitates the conservation of elite genotypes and accelerates breeding programs by producing large numbers of identical progenies (Anand et al., 2024). However, successful in vitro culture

depends heavily on optimizing the culture medium, particularly the basal nutrient formulation and plant growth regulators (PGRs), which regulate morphogenesis and organogenesis (Nandini et al., 2022). Basal media supply essential mineral nutrients, vitamins, and carbon sources, while PGRs—especially cytokinins and auxins—determine cell division patterns, shoot initiation, and proliferation rates (Abbas et al., 2023). Because plant species differ widely in their nutrient and hormonal requirements, empirical optimization is essential to achieve efficient shoot regeneration systems.

The Murashige and Skoog (MS) medium remains the most widely used basal formulation in plant tissue culture due to its balanced and relatively high levels of macronutrients and micronutrients (Murashige & Skoog, 1962). However, several studies have shown that reducing MS salt strength (½ MS) can improve explant growth and reduce tissue stress in some ornamentals, possibly by lowering osmotic potential and ionic toxicity (Sinha et al., 2022). The Hyponex medium, a fertilizer-based formulation, has also been used as a cost-effective alternative for in vitro propagation of ornamentals, particularly for rooting and hardening stages (Widowati et al., 2023). Nonetheless, the performance of these media can vary considerably depending on species and developmental stage, and comparative assessments for *A. plowmanii* shoot induction remain limited. Determining the most suitable basal medium is critical because it affects nutrient uptake, energy metabolism, and the responsiveness of explants to exogenous PGRs (Rahman et al., 2024).

Among PGRs, 6-Benzylaminopurine (BAP) is one of the most commonly used cytokinins for stimulating axillary bud proliferation and shoot regeneration in tissue culture (Kumar et al., 2023). BAP promotes cell division, delays leaf senescence, and breaks apical dominance, enabling dormant axillary buds to develop into multiple shoots (Ghosh et al., 2022). Numerous studies have reported that BAP supplementation increases the number of shoots per explant in various *Anthurium* species (Siregar et al., 2023). However, the optimal BAP concentration is highly genotype-specific; excessive BAP often leads to stunted shoot growth, hyperhydricity, or abnormal morphology (Alam et al., 2022). Furthermore, the response to BAP is strongly influenced by the basal medium composition, as nutrient levels affect cytokinin uptake, metabolism, and signaling (Silva et al., 2024). Therefore, assessing the interaction between basal media and BAP concentration is crucial to balance high shoot induction rates with acceptable shoot quality and vigor.

Despite growing commercial interest in *A. plowmanii*, systematic studies on the combined effects of basal media and BAP concentrations on its in vitro shoot multiplication are lacking. Previous work on other *Anthurium* spp. has shown that media composition significantly affects shoot regeneration efficiency, suggesting that genotype-specific optimization is required (Khatun et al., 2022). Developing an optimized shoot multiplication protocol would enable large-scale production of *A. plowmanii* plantlets, supporting both the ornamental plant industry and conservation of elite germplasm. Therefore, this study was conducted to evaluate the interactive effects of three basal media (MS, ½ MS, and Hyponex) and four BAP concentrations (0.00, 2.22, 6.66, and 13.32 µM) on shoot initiation, number of shoots, shoot height, and leaf number of *A. plowmanii* under in vitro conditions. The results are expected to provide a foundation for the development of efficient, scalable, and cost-effective propagation systems for this high-value ornamental species.

2. MATERIALS AND METHODS

2.1. Plant Material and Explant Preparation

Single-node stem segments of *Anthurium plowmanii* were used as explants. The source plants were obtained from healthy, uniform mother stock and subjected to surface sterilization. Explants were washed under running water, immersed in a mild detergent solution, and rinsed thoroughly. They were then disinfected with 70% ethanol for 30 seconds followed by 0.1% mercuric chloride for 5 minutes, and finally rinsed three times with sterile distilled water. The sterilized explants were aseptically trimmed to 1–1.5 cm in length and inoculated vertically onto the culture media.

2.2. Culture Media and Treatments

A factorial experiment was established using a Completely Randomized Design (CRD) with two factors: basal media and cytokinin concentration. The basal media treatments included full-strength Murashige and Skoog (MS), half-strength MS (½ MS), and Hyponex. Each basal medium was supplemented with one of four concentrations (0.00, 2.22, 6.66, and 13.32 µM) of 6-Benzylaminopurine (BAP). All media were supplemented with 30 g L^{-1} sucrose and solidified with 8 g L^{-1} agar, and the pH was adjusted to 5.8 before autoclaving at 121 °C for 15 minutes. Each treatment combination consisted of ten culture bottles containing one explant each, and all treatments were replicated three times.

2.3. Culture Conditions

All cultures were incubated in a growth room at 25 ± 2 °C under a 16-hour photoperiod provided by cool white fluorescent lamps with an intensity of approximately 40 µmol m^{-2} s^{-1}. Explants were observed daily for signs of contamination and initial shoot formation. Cultures were maintained for eight weeks, and subculturing was not performed during this period to allow evaluation of direct shoot induction and multiplication response.

2.4. Data Collection

Observations were recorded on: (1) days to shoot initiation, measured from the day of inoculation until visible bud emergence; (2) number of shoots per explant, counted at the end of the culture period; (3) average shoot height (cm), measured from the base to the tip of the tallest leaf; and (4) number of leaves per explant. Visual assessments were performed under a stereomicroscope to ensure accurate counting of small shoot initials. Data were recorded from all surviving explants in each treatment.

2.5. Statistical Analysis

All recorded data were subjected to two-way analysis of variance (ANOVA) to test the effects of basal medium, BAP concentration, and their interaction. When significant differences were detected at the 5% probability level, mean separation was carried out using Duncan's Multiple Range Test (DMRT). Statistical analysis was performed using SAS version 9.4, and results are presented as mean ± standard error.

3. RESULTS

3.1. Days to Shoot Initiation

All explants-initiated shoots within four weeks across treatments (Table 1). Basal media and 6-Benzylaminopurine (BAP) concentrations significantly affected the time to first bud emergence. The fastest initiation occurred on full Murashige and Skoog (MS) medium supplemented with 13.32 µM BAP (6.4 days) and on ½ MS with 13.32 µM BAP (6.8 days). In contrast, media without BAP delayed shoot emergence to over 15 days regardless of the basal medium.

Table 1: Effect of basal media and BAP concentration on days to shoot initiation of *Anthurium plowmanii*.

Basal Medium	BAP (µM)	Days to Shoot Initiation
MS	0.00	15.3
MS	2.22	10.1
MS	6.66	7.9
MS	13.32	6.4
½ MS	0.00	16.1
½ MS	2.22	11.2
½ MS	6.66	8.3
½ MS	13.32	6.8
Hyponex	0.00	18.4
Hyponex	2.22	13.5
Hyponex	6.66	10.2
Hyponex	13.32	8.7

Higher BAP levels accelerated shoot initiation across all media, with MS and ½ MS outperforming Hyponex. This suggests that the higher nutrient content of MS-based media synergizes with BAP to stimulate earlier meristem activation.

3.2. Number of Shoots per Explant

Shoot proliferation was strongly influenced by the interaction between basal media and BAP concentrations (Table 2). The highest shoot numbers were recorded on ½ MS + 13.32 µM BAP (10.7 shoots) and MS + 6.66 µM BAP (10.3 shoots). BAP-free media produced only 2–3 shoots on average.

Shoot multiplication increased with BAP concentration up to 6.66 µM but plateaued or slightly declined at 13.32 µM, suggesting that moderate cytokinin levels are optimal while excessive doses may inhibit further bud outgrowth.

3.3. Shoot Height

Shoot height was inversely related to BAP concentration (Table 3). The tallest shoots (3.9–4.2 cm) occurred on BAP-free media, while the shortest shoots (1.8–2.2 cm) occurred on media with 13.32 µM BAP. High BAP promoted shoot number but reduced elongation.

Table 2: Effect of basal media and BAP on shoot number of *Anthurium plowmanii*

Basal Medium	BAP (µM)	Shoots per Explant
MS	0.00	2.5
MS	2.22	6.1
MS	6.66	10.3
MS	13.32	9.5
½ MS	0.00	2.8
½ MS	2.22	6.8
½ MS	6.66	9.9
½ MS	13.32	10.7
Hyponex	0.00	1.9
Hyponex	2.22	4.3
Hyponex	6.66	7.2
Hyponex	13.32	8.0

Table 3: Effect of basal media and BAP on shoot height of *Anthurium plowmanii*

Basal Medium	BAP (µM)	Shoot Height (cm)
MS	0.00	4.1
MS	2.22	3.2
MS	6.66	2.4
MS	13.32	1.9
½ MS	0.00	4.2
½ MS	2.22	3.3
½ MS	6.66	2.5
½ MS	13.32	2.0
Hyponex	0.00	3.9
Hyponex	2.22	3.0
Hyponex	6.66	2.3
Hyponex	13.32	1.8

High cytokinin concentrations promoted more but shorter shoots, a common effect due to cytokinin-induced cell division outpacing elongation.

3.4. Number of Leaves per Explant

The number of leaves increased with BAP levels up to 13.32 µM, particularly on ½ MS medium (Table 4). The combination of ½ MS + 13.32 µM BAP yielded the highest mean leaf number (4.8).

BAP enhanced leaf initiation alongside shoot proliferation, and ½ MS supported more leaf formation than MS or Hyponex, indicating better nutrient balance for leaf morphogenesis.

3.5. Summary of Main Effects and Interactions

ANOVA revealed significant main effects of both basal medium and BAP concentration on all measured parameters and significant interactions for shoot number and leaf number (Figure 1-2, Table 5-6).

Both basal medium and BAP level significantly affected shoot multiplication traits, with the best balance of shoot number and quality achieved on ½ MS with 13.32 µM BAP.

Table 4: Effect of basal media and BAP on leaf number of *Anthurium plowmanii*.

Basal Medium	BAP (µM)	Leaves per Explant
MS	0.00	1.2
MS	2.22	2.7
MS	6.66	3.8
MS	13.32	4.1
½ MS	0.00	1.5
½ MS	2.22	2.9
½ MS	6.66	4.1
½ MS	13.32	4.8
Hyponex	0.00	1.1
Hyponex	2.22	2.2
Hyponex	6.66	3.1
Hyponex	13.32	3.7

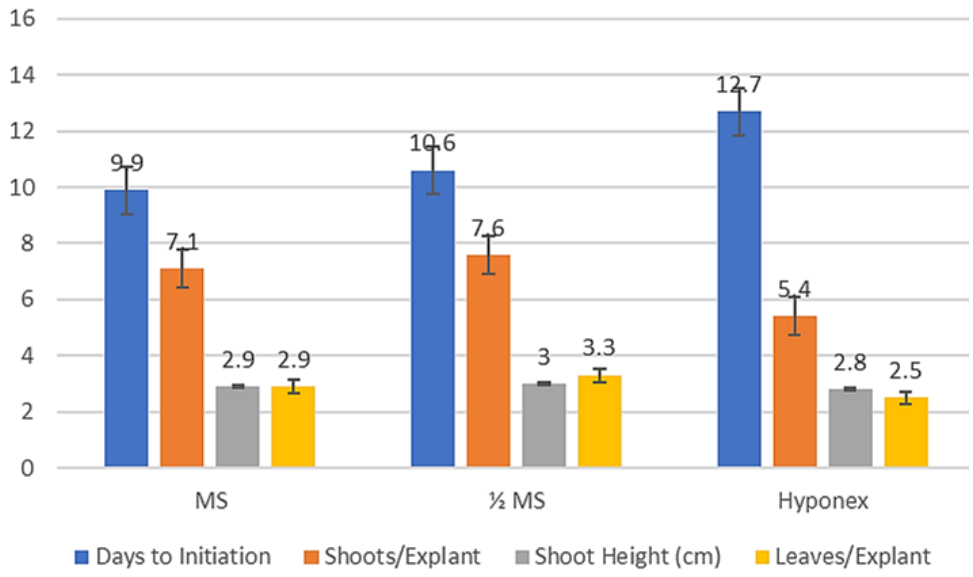

Fig. 1: Main effect of basal media on shoot induction responses (mean across BAP levels).

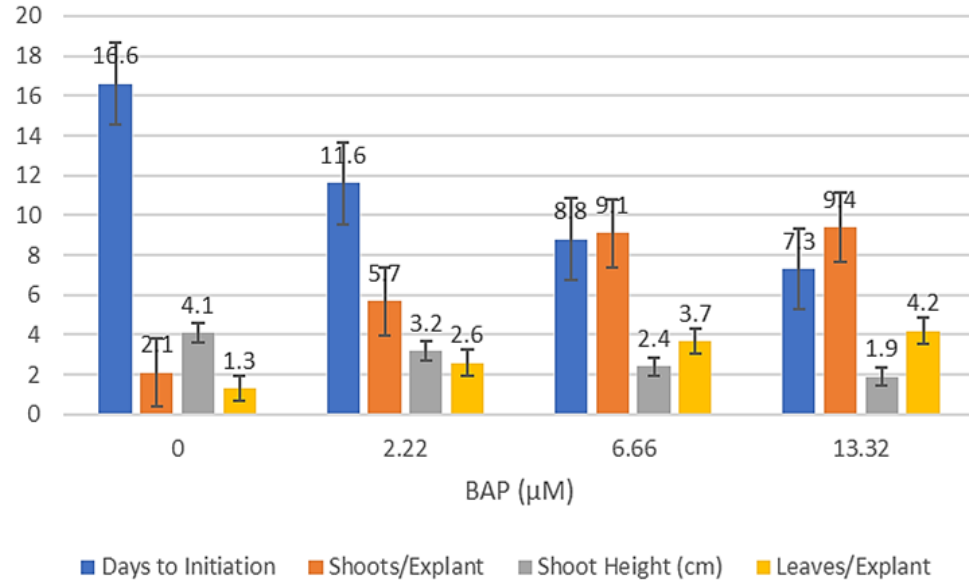

Fig. 2: Main effect of BAP on shoot induction responses (mean across media).

Table 5: ANOVA summary for basal media, BAP, and their interaction.

Parameter	Basal Media (p)	BAP (p)	Interaction (p)
Days to Initiation	<0.01	<0.01	0.07
Shoots per Explant	<0.01	<0.01	<0.05
Shoot Height	<0.01	<0.01	0.09
Leaves per Explant	<0.01	<0.01	<0.05

Table 6: Best-performing combinations ranked by overall performance.

Rank	Basal Medium	BAP (µM)	Shoot Number	Shoot Height (cm)	Leaves
1	½ MS	13.32	10.7	2.0	4.8
2	MS	6.66	10.3	2.4	3.8
3	MS	13.32	9.5	1.9	4.1
4	½ MS	6.66	9.9	2.5	4.1
5	Hyponex	13.32	8.0	1.8	3.7

4 | DISCUSSION

This study demonstrated that shoot induction and multiplication of *Anthurium plowmanii* were strongly influenced by the interaction between basal medium composition and the concentration of 6-Benzylaminopurine (BAP). Both

factors significantly affected days to shoot initiation, shoot number, shoot height, and leaf number, with ½-strength Murashige and Skoog (MS) medium supplemented with 13.32 µM BAP producing the highest number of shoots and leaves, while MS with 6.66 µM BAP supported rapid multiplication with moderate shoot elongation. The results underscore the importance of optimizing both nutrient supply and cytokinin levels to achieve high-efficiency micropropagation systems for *A. plowmanii*.

4.1. Influence of Basal Media Composition

The choice of basal medium had a pronounced impact on all growth parameters, confirming that mineral nutrient composition is a critical determinant of in vitro morphogenesis. Explants cultured on full-strength MS produced shoots faster and with greater vigor than those on Hyponex medium, consistent with the high macronutrient content of MS, which promotes cell division and protein synthesis (Rahman et al., 2024). However, ½ MS generally outperformed full-strength MS in terms of shoot number and leaf development. This aligns with findings that reducing the salt strength of MS can lower osmotic stress and ionic toxicity, creating a more favorable environment for meristematic activity (Sinha et al., 2022). Several studies have reported similar responses in other Anthurium species, where ½ MS promoted higher shoot multiplication than full MS (Khatun et al., 2022; Ghosh et al., 2022).

The poorer performance of Hyponex medium in this study likely reflects its lower micronutrient and nitrogen content, which may limit cell proliferation during the early stages of shoot induction. Although Hyponex-based formulations are often used as cost-effective rooting media for ornamentals (Widowati et al., 2023), they may be suboptimal for the active cell division and organogenesis required during shoot multiplication. This supports the view that media rich in nitrate nitrogen, such as MS, are more suitable for cytokinin-driven shoot proliferation (Silva et al., 2024). The significant main effect of basal medium detected in the ANOVA confirms that nutrient supply independently affects morphogenesis regardless of cytokinin levels.

4.2. Role of BAP in Shoot Induction

BAP is a synthetic cytokinin widely used to stimulate axillary bud outgrowth and shoot multiplication. In this study, increasing BAP concentration reduced the time to shoot initiation and increased the number of shoots and leaves per explant, confirming its strong mitotic activity. Cytokinins promote cell cycle progression by upregulating cyclin-dependent kinases and downregulating genes associated with apical dominance, thereby activating dormant axillary meristems (Kumar et al., 2023). The positive response of *A. plowmanii* to BAP is consistent with results in other *Anthurium* spp., such as Anthurium andreanum and Anthurium crystallinum, where BAP stimulated rapid shoot bud proliferation (Siregar et al., 2023; Putri et al., 2024).

However, shoot height decreased as BAP concentration increased, a pattern commonly observed in cytokinin-rich media. High cytokinin levels promote cell division over cell elongation, resulting in compact shoots with shorter internodes (Alam et al., 2022). This effect is generally desirable during the multiplication phase because it produces multiple shoot initials from limited explant tissue, but excessive cytokinin can cause hyperhydricity, fasciation, or morphological abnormalities (Mhatre et al., 2023). In this study, the highest shoot numbers were obtained at 6.66–13.32 µM BAP, while shoot elongation was optimal at lower concentrations, suggesting a trade-off between proliferation rate and shoot quality. This supports the common micropropagation practice of using high-cytokinin media for shoot induction followed by transfer to lower-cytokinin or cytokinin-free media for elongation and rooting (Abbas et al., 2023).

4.3. Interaction Between Basal Media and BAP

A key finding was the significant interaction between basal media and BAP concentration, especially for shoot and leaf numbers. ½ MS with 13.32 µM BAP produced the most shoots (10.7 per explant) and leaves (4.8 per explant), outperforming full-strength MS at the same BAP level. This suggests that lowering the ionic concentration of the basal medium enhanced the responsiveness of *A. plowmanii* explants to BAP. Similar synergistic effects between reduced-strength media and cytokinins have been reported in *Anthurium andreanum* (Khatun et al., 2022) and *Spathiphyllum wallisii* (Rani et al., 2024). High total salt concentrations can inhibit cytokinin uptake or induce osmotic stress, while moderate nutrient levels can favor cytokinin-mediated organogenesis (Nandini et al., 2022). The present findings confirm that adjusting basal nutrient strength can improve cytokinin efficiency, likely by optimizing the cellular redox balance and carbohydrate metabolism that support active meristem proliferation (Silva et al., 2024).

The interaction also highlights the genotype-specific nature of tissue culture responses. While full-strength MS is often considered the default medium, its performance varies across species and even among cultivars within a species. This reinforces the need for empirical optimization of both basal media and PGR regimes for each target species

(Chakraborty et al., 2023). In *A. plowmanii*, moderate nutrient strength combined with relatively high cytokinin appears to best stimulate shoot organogenesis, whereas high nutrients plus high cytokinin produced slightly fewer shoots, possibly due to feedback inhibition or nutrient–hormone imbalances.

4.4. Comparison with Previous Studies in *Anthurium* spp.

The results are broadly consistent with earlier reports on *Anthurium* micropropagation. Khatun et al. (2022) found that ½ MS medium with 13.3 µM BAP induced up to 11 shoots per explant in *A. andreanum*. Siregar et al. (2023) also reported that BAP concentrations of 6.7–11 µM maximized shoot proliferation but reduced shoot length, similar to this study. However, the relatively high shoot numbers obtained here suggest that *A. plowmanii* may be more responsive to cytokinin stimulation than some other *Anthurium* species, highlighting its high regeneration potential. This is advantageous for commercial propagation but also underscores the need to monitor for cytokinin-induced abnormalities during scaling up.

While Hyponex medium was less effective overall, its performance improved at higher BAP levels, suggesting that hormonal stimulation can partially compensate for lower nutrient content. Widowati et al. (2023) noted that Hyponex-based media can support limited shoot proliferation if supplemented with cytokinins, though growth is slower than on MS-based media. These findings collectively indicate that for large-scale production, MS-based formulations remain superior for multiplication stages, while Hyponex may be better suited for later rooting or acclimatization stages where lower nutrient strength is advantageous.

4.5. Implications for Commercial Micropropagation

The development of an efficient shoot multiplication protocol is critical for meeting the increasing demand for *A. plowmanii* plantlets. High shoot numbers per explant (≥10) within a single culture cycle, as achieved in this study, can dramatically increase production efficiency and reduce the number of subculture cycles needed, minimizing labor and contamination risks (Anand et al., 2024). Moreover, using ½ MS medium can lower media costs and reduce tissue stress while maintaining high regeneration rates, offering economic and physiological advantages for commercial propagation systems. Incorporating such optimized protocols into nursery production could support the rapid scaling of *A. plowmanii* while preserving elite genotypes.

This study also contributes to broader efforts in optimizing micropropagation of ornamental aroids, many of which face similar propagation constraints. The demonstrated synergy between basal nutrient strength and cytokinin level can serve as a model for protocol development in other species. Efficient in vitro systems not only support commercial horticulture but also aid in the conservation of rare or endangered ornamental genotypes by enabling ex situ germplasm maintenance and distribution (Chakraborty et al., 2023).

4.6. Future Directions

Although the current findings provide a strong foundation for *A. plowmanii* micropropagation, several refinements could further enhance efficiency. Future work should evaluate the addition of low levels of auxins such as indole-3-acetic acid (IAA) or naphthaleneacetic acid (NAA) alongside BAP, as auxin–cytokinin balance influences not only shoot proliferation but also subsequent rooting (Rani et al., 2024). It will also be useful to assess the effects of different gelling agents and carbohydrate sources on shoot quality, as these factors can affect water availability and energy metabolism in vitro (Abbas et al., 2023). Genetic fidelity of regenerated plantlets should be confirmed using molecular markers such as SSRs or RAPDs to ensure clonal uniformity, which is essential for commercial production (Gómez et al., 2023). Finally, acclimatization success and ex vitro performance of regenerated plantlets need evaluation to confirm that high shoot multiplication rates translate into healthy, vigorous plants.

5. Conclusion

This study demonstrated that the interaction between basal medium composition and 6-Benzylaminopurine (BAP) concentration plays a pivotal role in regulating in vitro shoot induction of *Anthurium plowmanii*. Among the tested combinations, ½-strength Murashige and Skoog (MS) medium supplemented with 13.32 µM BAP produced the highest number of shoots and leaves, while MS with 6.66 µM BAP supported rapid multiplication with moderate shoot elongation. High BAP levels accelerated shoot initiation and enhanced shoot and leaf production but suppressed shoot elongation, whereas low BAP promoted elongation but resulted in fewer shoots. The superior performance of ½ MS indicates that lowering salt strength enhances explant responsiveness to cytokinin by minimizing ionic stress. These findings provide an efficient and reproducible shoot multiplication protocol for *A. plowmanii*, which can support large-scale commercial propagation and conservation of elite germplasm.

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Data Availability: The data collected for this article are included in the article.

Ethics Statement: No prior study was conducted on live animals/humans; thus, it did not require any ethical approval.

Authors' Contribution: YS designed the study and structured the manuscript outline, contributed to the data evaluation and prepared the manuscript and approved the final version of the manuscript.

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