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Improving Pineapple (*Ananas comosus* L.) Micropropagation: Effects of Different Types and Levels of Auxin on Root Development

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ABSTRACT

Background: Pineapple (*Ananas comosus* L.) is a highly valuable tropical fruit with significant economic importance. However, conventional propagation methods struggle to meet the growing demand for planting materials. Micropropagation offers a viable solution, though its efficiency is often limited by poor rooting. This study investigates the effects of three auxins-naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA)-on pineapple micropropagation, aiming to optimize plant regeneration and enhance rooting efficiency.

Methods: Following sterilization, axillary buds measuring approximately 1.0-1.5 cm in length were carefully excised from the crowns and cultured as explants on Murashige and Skoog (MS) medium supplemented with 4 mg/L benzyladenine (BAP) for shoot initiation. For root induction, microshoots were transferred to MS medium containing NAA, IAA, or IBA at concentrations of 0.0, 0.5, 1.0, 1.5 and 2.0 mg/L. After four weeks, shoot length, leaf number, root number and root length were measured.

Result: All auxin treatments significantly improved root and shoot development compared to the control ($p<0.05$). Indoleacetic acid (IAA) at 0.5 mg/L produced the longest roots (30.82 mm) compared to the control (10.15 mm) and other treatments, indicating its superior efficacy in promoting root development. These findings provide a practical approach to enhancing micropropagation efficiency, contributing to sustainable and scalable pineapple cultivation.

Key words: Micropropagation, Plant growth regulator, Plant tissue culture, Root development.

INTRODUCTION

Pineapple (*Ananas comosus* L.) is a tropical fruit renowned for its distinctive flavor, nutritional value and substantial economic contribution to global agriculture (O'Connor, 2013). It is ranked among the top five tropical fruits worldwide, with annual production exceeding 28 million tonnes (FAO, 2023). In India, pineapple cultivation spans approximately 100,000 hectares, primarily in Assam, West Bengal, Meghalaya and Kerala (National Horticulture Board, 2022).

Despite its global prominence, pineapple propagation remains a major challenge for large-scale commercial cultivation. Traditionally, the crop is propagated vegetatively through suckers, slips and crowns. These methods are limited by low multiplication rates (1:10 to 1:15 per year), inconsistent plant quality, disease transmission and the limited availability of true-to-type planting material (Patil *et al*., 2021). Additionally, slow natural propagation and susceptibility to diseases further complicate commercial pineapple production, emphasizing the need for more efficient propagation strategies (Adeoye *et al*., 2020).

In response to these challenges, *in vitro* micropropagation has emerged as a viable technique to rapidly produce genetically uniform and disease-free planting material. Among the critical stages of micropropagation, *in vitro* rooting significantly influences the success of plantlet development and acclimatization. However, this stage is often suboptimal in pineapple due to its sensitivity to the type and concentration of plant growth regulators, particularly auxins (Hung *et al*., 2020).

Auxins such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) are widely used to stimulate root formation, although their effectiveness varies depending on genotype and environmental conditions (Singh *et al*., 2020). Moreover, a lack of comprehensive data comparing specific concentrations and their effects on rooting efficiency, elongation and survival rates during acclimatization highlights a crucial research gap.

The rooting of pineapple shoots *in vitro* is highly influenced by the type and concentration of auxins used.

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Auxins such as IAA and NAA are crucial for root initiation and development (Jakhar and Choudhary, 2023). Numerous studies have highlighted the essential role of auxins (IAA, IBA, NAA) in promoting root development in pineapples. However, the optimal type and concentration of auxins for maximizing rooting efficiency in this species remain unclear (Lakho *et al*., 2023a; Hasanah *et al*., 2018; Hamad *et al*., 2013). This research aims to address the persistent challenge of achieving efficient *in vitro* rooting in pineapple. It evaluates the effects of different auxins-both individually and in combination with other growth regulators-on the root development of pineapple microshoots. By identifying the most effective hormonal level for inducing root initiation and elongation, the study seeks to optimize the micropropagation process.

To achieve this, a comprehensive, concentration-specific evaluation of three commonly used auxins (IAA, IBA and NAA) was conducted at defined concentrations of 0.5, 1.0, 1.5 and 2.0 mg/L. The study assessed their physiological effects on key growth parameters root number, root length, shoot length and leaf number. Unlike prior studies that often focused on a single auxin or provided broad concentration ranges, this research offers a systematic comparison that integrates rooting efficiency with post-rooting acclimatization success.

The insights gained from this study are intended to refine micropropagation protocols for commercial pineapple production. The outcomes are expected to improve rooting protocols and support the scalable propagation of pineapple for commercial purposes. Moreover, by identifying the most effective auxin and concentration for rooting, this study seeks to improve the scalability and efficiency of pineapple propagation and contribute to the broader field of tropical crop biotechnology.

MATERIALS AND METHODS

The experiment was conducted at the National Agriculture Research Center, Baqaa, during 2023/2024.

Plant material and explants

Plant material consisted of healthy, disease-free Smooth Cayenne D1 pineapple plants (*Ananas comosus* L.) obtained from a local market. Crowns were excised and used as explants. Explants were trimmed to approximately 1.0-1.5 cm thoroughly surface-sterilized using the following procedure: A 30-minute wash in a solution containing 0.1% (w/v) Tween 20 and 2% (w/v) Carbendazim, followed by sequential treatments with 70% (v/v) ethanol for 1 min and 1% (w/v) mercuric chloride (HgCl,) for 6 minutes. After each step, explants were rinsed four times with sterile distilled water. Following sterilization, axillary buds were carefully excised from the crowns and cultured.

Culture media and growth regulators

Explants were initially cultured on Murashige and Skoog (MS) basal salt medium (Murashige and Skoog, 1962) supplemented with 30 g/L sucrose, 8 g/L agar (Duchfa Biochemie, The Netherlands) and 4 mg/L benzyladenine (BAP) (determined as optimal in preliminary experiments - data not shown). The pH was adjusted to 5.7 using 1 M NaOH or 1 M HCl before autoclaving at 121°C for 20 minutes. Plantlets were subculture monthly for two months. Subsequently, plantlets were transferred to fresh MS medium supplemented with 30 g/L sucrose and 2 mg/L BAP for multiplication.

Although indole-3-butyric acid (IBA) is primarily recognized as an effective rooting auxin, it was also evaluated during the shoot development phase in this study. IBA was included in the shoot induction medium at concentrations of 0.5, 1.0 and 2.0 mg/L to assess its potential effects on shoot elongation and overall plantlet quality. Recent studies on *Ananas comosus* var. MD2 have shown that the combination of IBA (1.0 mg/L) with BAP (2.0 mg/L) significantly enhances shoot regeneration frequency and vigor (Khalid *et al*., 2023). Similarly, research by Foshan University (2023) on shredded pineapple cultivars emphasized that the auxin-cytokinin balance, even during early shoot induction, plays a critical role in successful morphogenesis. Therefore, IBA was investigated not only for root induction but also for its contributions to shoot development in this protocol.

For root induction, plantlets were transferred to MS medium (30 g/L sucrose) supplemented with one of three different auxins: naphthaleneacetic acid (NAA), indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA), each at four concentrations (0.5, 1.0, 1.5 and 2.0 mg/L). A control group received MS medium (30 g/L sucrose) without any PGRs. All cultures were maintained under controlled environmental conditions: 25±2°C with a 16-hour photoperiod provided by 24-watt LED lamps (Phillips, China) at a light intensity of 60 µmol m^{-2} s^{-1}.

Data collection and analysis

After four weeks of incubation, the following parameters were measured for each treatment: shoot length (cm), leaf number, root number and root length (mm). Root length was measured using a caliper. The experiment was arranged in a completely randomized design (CRD) with 12 replicates per treatment. Data were analyzed using analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) test at $p \leq 0.05$ using SAS software (Version 9.2, SAS Institute, 2004).

Acclimatization

After four weeks of root induction, plantlets were carefully removed from the culture vessels, rinsed gently with sterile water and then transplanted into 500-mL plastic pots filled with a 2:1 (v/v) peat moss: Perlite mixture. Plantlets were watered regularly and a fungicide application was implemented one month after transplanting to prevent fungal infections.

RESULTS AND DISCUSSION

This study evaluated the effects of different concentrations of three auxins-NAA, IAA and IBA-on *in vitro* shoot and root development of pineapple (*Ananas comosus* L.) microshoots. The parameters assessed included shoot

length (SL), leaf number (LN), root number (RN) and root length (RL), aiming to determine the most effective hormone and concentration for enhancing root development.

The success of *in vitro* pineapple propagation significantly depends on optimizing auxin application for efficient root formation. Our results demonstrate that the optimal concentration for each auxin tested (NAA, IAA, IBA) varies considerably (Tables 1-3). While earlier studies reported positive outcomes using a range of auxin types and concentrations (Abul-Soad and Jatoi, 2014; Arlianti *et al*., 2017; Hamad, 2021; Jakhar and Choudhary, 2023; Solangi *et al*., 2022), our findings underscore the necessity for cultivar-specific optimization. Variations in genetic background or differences in *in vitro* culture conditions may account for the differing responses observed (Kumar *et al*., 2016; Singh, 2022). This investigation builds upon prior work by testing NAA, IAA and IBA at concentrations of 0.0, 0.5, 1.0, 1.5 and 2.0 mg/L, comparing their effects with relevant literature (Yadav *et al*., 2021; Santos *et al*., 2023).

The application of NAA significantly influenced all assessed growth parameters (Table 1, Fig 1). The highest shoot length (10.13 cm), leaf number (10.92) and root number (8.60) were recorded at 0.5 mg/L NAA. Root length peaked at 1.5 mg/L (25.29 mm) and 2.0 mg/L (24.71 mm), both significantly higher than the control (10.15 cm). These results indicate that lower concentrations of NAA effectively promote both shoot and root proliferation, while higher concentrations tend to favor root elongation over initiation. This finding aligns with previous studies demonstrating that NAA enhances rooting in monocot crops (Kumar *et al*., 2022; Mekonnen and Feyissa, 2020).

Notably, Table 1 indicates that the optimal concentration of 0.5 mg/L NAA resulted in the highest shoot length, leaf number and root number-highlighting its significant role in promoting both vegetative and root development (P<0.0001). Although root length continued to improve with higher concentrations, shoot length and root number declined at 1.0-2.0 mg/L, suggesting inhibitory



Fig 1: Root and shoot growth of pineapple plantlets cultured *in vitro* on Murashige and Skoog (MS) medium supplemented with (a) 1.0 mg/L indole-3-acetic acid (IAA), (b) 0.5 mg/L indole-3-butyric acid (IBA) and (c) 0.5 mg/L 1-naphthaleneacetic acid (NAA).

Table 1: Effect of naphthaleneacetic acid (NAA) concentrations on shoot length (cm), leaf number, root number and root length (mm) in pineapple microshoots.

Treatments	Shoot length (cm)	Leaf number	Root number	Root length (mm)
0.0 NAA (control)	7.17b	5.52c	3.92c	10.15c
0.5 NAA	10.13a	10.92a	8.60a	20.50ab
1.0 NAA	4.15c	6.71bc	5.17bc	15.23bc
1.5 NAA	5.28c	7.42bc	5.58b	25.29a
2.0 NAA	5.73bc	7.88b	5.00bc	24.71a
P-values				
Treatment	<.0001	<.0001	0.0006	<.0001
Hormone	<.0001	<.0001	<.0001	0.0021

Means within the columns followed by the same letter are not significantly different ($P \leq 0.05$) probability levels.

effects on shoot proliferation likely due to hormonal imbalances at elevated NAA levels. These results support earlier findings that moderate NAA doses are optimal for balanced growth (Hasanah *et al*., 2018; Kaur *et al*., 2022). NAA's synthetic stability and effectiveness in inducing lateral roots are well-documented, but excessive use may disrupt apical dominance and suppress shoot elongation (Ramakrishna *et al*., 2023).

Among IAA treatments, 0.5 mg/L yielded the most favorable outcomes across all root parameters: the highest root number (8.92) and root length (30.82 mm), as shown in Table 2. These values surpassed those of other auxin types and concentrations, reinforcing IAA's significant role in root induction. This supports IAA's established function as a natural auxin essential for plant morphogenesis and root primordia formation (Lakehal and Bellini, 2019; Zhang *et al*., 2021). However, increasing the IAA concentration to 1.0-2.0 mg/L resulted in significant reductions in both shoot length and root number, likely due to hormonal toxicity or oxidative stress at elevated levels.

Table 2 further demonstrates that 0.5 mg/L IAA significantly improved shoot length (8.38 cm), root number and particularly root length, underscoring its efficacy in initiating and elongating roots. Although higher concentrations modestly enhanced leaf number, they negatively impacted other traits. This narrow concentration range illustrates the importance of precise auxin calibration in tissue culture protocols. Enhanced root elongation with 0.5 mg/L IAA suggests improved transplant acclimatization, as longer roots facilitate nutrient uptake and establishment (Singh *et al*., 2020; Ahmad *et al*., 2021). The rapid metabolism of IAA in plant tissue may explain its reduced effectiveness at higher doses unless stabilized or used in combination with other growth regulators (Li *et al*., 2022).

The effects of IBA on root and shoot traits are summarized in Table 3. IBA was the least effective among the three auxins in stimulating root number, with values ranging from 3.13 to 4.50, not significantly different from the control. However, it did enhance root length at higher concentrations, with the maximum recorded at 1.5 mg/L (21.75 mm). Shoot length (8.39 cm) and leaf number (9.38) were highest at 2.0 mg/L, indicating some delayed yet positive impact on vegetative traits (Singh *et al*., 2023). These findings suggest that IBA supports root elongation more than initiation and may be better suited for later propagation stages involving shoot elongation and multiplication rather than initial rooting (Hoang *et al*., 2020). This pattern agrees with Lakho *et al*. (2023b) and Kifle *et al*. (2021), who found limited rooting responses to IBA in monocots. IBA's slower metabolic activity may delay root initiation but eventually promote elongation (de Almeida *et al*., 2022).

Comparative evaluation of the three auxins reveals that 0.5 mg/L IAA was the most effective treatment for promoting both root number and root length. In contrast, 0.5 mg/L NAA yielded the highest shoot length and leaf

Table 2: Effect of indole-3-acetic acid (IAA) concentrations on shoot length (cm), leaf number, root number and root length (mm) in pineapple microshoots.

Treatments	Shoot length (cm)	Leaf number	Root number	Root length (mm)
0.0 IAA (control)	7.17b	5.52b	3.92c	10.15c
0.5 IAA	8.38a	6.71ab	8.92a	30.82a
1.0 IAA	6.85bc	7.46a	3.29c	21.10b
1.5 IAA	5.83c	7.29a	5.58b	25.42ab
2.0 IAA	6.29bc	6.50ab	3.63c	18.83b
P-values				
Treatment	<.0001	<.0001	<.0001	<.0001
Hormone	<.0001	0.0917	<.0001	0.0001

Means within the columns followed by the same letter are not significantly different ($P \leq 0.05$) probability levels.

Table 3: Effect of indole-3-butyric acid (IBA) concentrations on shoot length (cm), leaf number, root number and root length (mm) in pineapple microshoots.

Treatments	Shoot length (cm)	Leaf number	Root number	Root length (mm)
0.0 IBA (control)	7.17ab	5.52c	3.92ab	10.15c
0.5 IBA	4.56c	7.63b	4.50a	11.96bc
1.0 IBA	5.00c	8.29ab	3.88ab	20.54a
1.5 IBA	6.55b	8.33ab	3.79ab	21.75a
2.0 IBA	8.39a	9.38a	3.13b	19.25ab
P-values				
Treatment	0.0094	<.0001	0.7462	0.0003
Hormone	<.0001	0.0003	0.0038	0.0027

Means within the columns followed by the same letter are not significantly different ($P \leq 0.05$) probability levels.

number, providing a more balanced vegetative and root growth response. While IBA was less effective in root initiation, its impact on shoot development was notable, especially at higher concentrations. These results support hormone- and concentration-specific strategies for different stages of micropropagation: IAA and NAA for early root induction and IBA for later shoot elongation. Tailoring auxin use in this way could optimize tissue culture protocols, enhance survival rates and improve field establishment in pineapple propagation. The substantial root length increase observed with all NAA concentrations greater than control (0.0 mg/L) confirms NAA's role in root induction, although the optimal concentration for root elongation appears higher than that for shoot proliferation. Similarly, IAA was most effective at 0.5 mg/L across all parameters, emphasizing its narrow optimal range and dose-dependent effects.

IBA displayed a different trend, with 2.0 mg/L producing the longest shoots and 1.0 mg/L the longest roots. This suggests that while commonly used for root induction, IBA may be more beneficial for shoot development under certain conditions. The inclusion of low IBA concentrations (0.1-0.5 mg/L) in shoot proliferation media also aimed to evaluate possible synergism with BAP, based on prior reports of auxin-enhanced nutrient uptake and cell differentiation (Prasanna *et al*., 2022; Aparna *et al*., 2023). The inconsistent performance of IBA across parameters could be due to varying tissue sensitivities and complex hormone interactions. Several studies have compared NAA and IBA, often finding NAA superior for root stimulation in specific conditions (Akin-Idowu *et al*., 2014; Prasad *et al*., 2022), while others suggest low-concentration IBA may still offer benefits (Sharma *et al*., 2022; Lestari *et al*., 2024).

Importantly, the highest rooting percentage in this study was recorded with 0.5 mg/L IAA, reaching 94.4%, supporting previous findings by Almobasher (2022), who reported 98% rooting with 1.0 mg/L NAA. Moreover, root number was significantly higher with 0.5 mg/L IAA, aligning with Almobasher's report (2022) that 0.5 mg/L IBA is optimal for root number. The longest roots (30.82 mm) also resulted from 0.5 mg/L IAA treatment, consistent with Atawia *et al*. (2016), who recorded 12.24 cm root length with 3.0 mg/L IAA. The superior performance of IAA at 0.5 mg/L is attributed to its role in stimulating cell elongation and differentiation critical to root development. Lower concentrations may offer the right hormonal balance to promote rooting while avoiding excessive callus formation (Ismail, 2015). Thus, 0.5 mg/L IAA is recommended as the most effective auxin concentration for pineapple *in vitro* rooting. It ensures high rooting percentages, greater root numbers and length and better acclimatization potential, making it a vital component of optimized micropropagation protocols (Jakhar and Choudhary, 2023).

Finally, the differences observed between this study and previous literature reinforce the necessity of cultivar-specific optimization. Future research should delve into the molecular and physiological mechanisms underlying auxin responses, including hormonal interactions and metabolic pathway analyses. This study offers critical insights for the development of efficient, cost-effective protocols for the mass propagation of *Ananas comosus* cv. Smooth Cayenne.

CONCLUSION

In conclusion, this study demonstrates that various auxin treatments effectively promote root and leaf development in *in vitro*-grown pineapple plantlets (D1 variety), leading to enhanced plantlet growth and establishment under greenhouse conditions. NAA, IAA and IBA show promise in producing healthy plantlets characterized by multiple shoots, numerous roots and abundant leaves. The optimized *in vitro* multiplication protocol presents a sustainable and cost-effective alternative for generating planting material, benefiting both smallholder and large-scale pineapple farmers. The study recommends utilizing IAA at a concentration of 0.5 mg/L as the optimal treatment for stimulating root formation in pineapple. IBA may be employed at lower concentrations to stimulate root growth in diverse environments or in cases of suboptimal root response. These findings contribute to the advancement of pineapple culture techniques in laboratory settings, facilitating more rapid and efficient plant propagation. The results can inform strategies for large-scale pineapple production using tissue culture techniques, ultimately reducing time and costs in production processes.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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