Original Research Article

Aloe vera Gel as a Substitute for Commercial Auxins in the *in-vitro* Culture of Sour Banana (*Mysore AAB)*

ABSTRACT

Banana (*Musa* spp.) is an important tropical fruit crop known for its economic and nutritional value, and the sour banana variety *Mysore AAB* is highly valued in Sri Lanka for its taste, yield, low price and storage characteristics. Although plant growth regulators, especially auxins like Indole-3-acetic acid (IAA), are essential for the initiation of roots and development of shoots in tissue culture; these synthetic auxins increase production costs and limit accessibility. Therefore, the current study was conducted at Plant Tissue Culture Research and Development Laboratory of the University of Colombo Institute for Agro-Technology and Rural Sciences, Sri Lanka, from May to October 2024, with the objective of investigating the potential of *Aloe vera* gel (AVG) as a substitute for IAA in the *in-vitro* culture of banana. Explants were cultured on Murashige and Skoog (MS) medium supplemented with varying concentrations of AVG (120, 160, 200, 240, 280, 360, 440 and 520 g/L) and compared with a control containing 0.1 mg/L IAA. Number of roots, shoot height (mm), root dry weight (g) and shoot dry weight (g) were collected over a period of 6 weeks and the collected data were subjected to analysis of variance and mean separation using Tukey's test, at 5% significance level. Results showed that, compared to the control, treatments containing AVG initially produced significant differences in root number among the treatments, however these differences diminished by the sixth week ($p>0.05$). A comparable pattern was noted in the root dry weight which produced no significant differences (($p>0.05$) among the treatments. Although the shoot height at 6th week showed significant differences (($p<0.05$) among treatments, most treatments performed similar to the control (45.9 mm), indicating AVG as an effective alternative to auxins. Shoot dry weight also showed a similar trend, accumulating a comparable shoot biomass in the control, with the treatments containing AVG. These findings emphasize *Aloe vera* gel as a cost-effective, accessible, and sustainable choice for the micropropagation of sour banana (*Mysore AAB*).

Keywords: Aloe vera gel, auxins, IAA, banana, micropropagation

1. INTRODUCTION

Banana (*Musa* spp.) belonging to family Musaceae, ranks as the fifth most significant agricultural food crop in international trade (Singh et al., 2016). It is widely cultivated in the tropical and subtropical regions of the world and plays a vital role in food security, nutrition, and income generation. Being a tasty and favored fruit enjoyed by individuals of all ages, bananas are eaten in a variety of both dry and fresh forms (Al-Dairi et al., 2023). In Sri Lanka, banana has become the most important fruit crop in terms of production, extent of cultivation and consumption, as it offers year-round financial benefits to farmers (Kudagamage, 2004; Hathurusinghe et al., 2012). In the local market, there is a high demand for varieties such as 'Ambul' (sour banana), 'Ambun' (Cavendish), 'Kolikuttu' (silk banana), 'Seeni' (sugar banana), 'Puwalu', and 'Anamalu', of which 'Ambul' (sour banana) is less expensive than the others (Kudagamage, 2004; Perera et al., 1999). The sour banana variety *Mysore AAB,* which has a distinctive flavor, is widely grown within the country in various agro-ecological zones, due to its high yield and good keeping quality (Sirisena & Senanayake, 1997). However, conventional propagation of banana through suckers or corms is constrained by low

multiplication rates, seasonal availability, and the risk of transmitting pests and diseases (Al-Amin et al., 2009; Gübbük & Pekmezci, 2004). As a result, *in-vitro* propagation has become a widely adopted technique for the quick, year-round production of uniform, disease-free planting material with early growth and shorter harvest intervals (Gübbük & Pekmezci, 2004). which is attempted in the present study.

Plant growth regulators, particularly auxins and cytokinins, are key components of tissue culture media as they regulate root initiation, cell division, and cell elongation, thereby playing a central role in overall plantlet development (Mohamad et al., 2022; Pasternak & Steinmacher, 2024). Therefore, selecting the right combination and concentration of these regulators is critical for the success of tissue culture protocols. Out of many auxins available, Indole-3-acetic acid (IAA) is one of the most commonly used auxins in banana micro propagation. Despite its effectiveness, the use of synthetic auxins increases production costs and may limit accessibility for laboratories and farmers with limited resources. This has generated a growing interest in finding natural and affordable substitutes for commercial plant growth regulators that can promote sustainable practices in plant tissue culture. One such example is *Aloe vera* gel, which is a natural plant extract, rich in vitamins, amino acids, lipids, enzymes, carbohydrates, minerals, and phytohormones such as auxins and gibberellins (Mirihagalla & Fernando, 2020; Gantait et al., 2014). Previous studies have also reported that *Aloe vera* gel can positively influence plant growth, rooting, and regeneration in different plant species, pointing to its possibility to be used as a natural alternative to synthetic growth regulators in *in-vitro* culture systems (Hamdeni et al., 2022; Kumari et al., 2023). Moreover, *Aloe vera* is cost-effective, eco-friendly, and readily available in Sri Lanka, which makes it an appealing option for application in plant tissue culture.

Considering the economic significance of sour banana and the necessity for sustainable micropropagation methods, this research was conducted to examine the effectiveness of *Aloe vera* gel as an alternative to commercial auxins in the *in-vitro* culture of sour banana (*Mysore AAB*). Specifically, the study aimed to evaluate the impact of varying concentrations of *Aloe vera* gel on the root and shoot development of banana explants in relation to the conventional IAA-supplemented Murashige and Skoog (MS) medium.

2. MATERIALS AND METHODS

2.1 Experimental Location and Period

This study was conducted at the Plant Tissue Culture Research and Development Laboratory of the University of Colombo Institute for Agro-Technology and Rural Sciences (UCIARS), Sri Lanka, from May to October 2024.

2.2 Preparation of Aloe vera gel

Mature *Aloe vera* leaves needed for the research were collected from Weligatta area, in Hambantota district. The plant material was thoroughly rinsed with tap water to eliminate soil residues, followed by surface cleaning with Tween-20 (a non-ionic detergent) and 70% ethanol. Leaf peels and side thorns were discarded, and the leaves were subsequently homogenized using a blender without the addition of water, to prepare the *Aloe vera* gel (AVG). It was then filtered using a double-layer muslin cloth to obtain pure gel.

2.3 Preparation of MS medium

Murashige and Skoog (1962) medium was used for the experiment. Indole-3-Acetic Acid and *Aloe vera* gel was added to the MS medium based on the treatments of the experiment (Table 1).

2.4 Experimental Design and Treatments

The experiment followed a Completely Randomized Design (CRD), consisting of nine treatments (Table 2), each replicated fifteen times.

Table 1. Treatments of the experiment.

Treatment Code	Concentration added to MS medium
T1 (Control)	0.1 mg/L IAA
T2	120 g/L AVG
T3	160 g/L AVG
T4	200 g/L AVG
T5	240 g/L AVG
T6	280 g/L AVG
T7	360 g/L AVG
T8	440 g/L AVG
T9	520 g/L AVG

IAA = Indole-3-Acetic Acid; AVG = *Aloe vera* gel

2.5 Selection, Sterilization, and Culture of Explants

Young sour banana ex-plants were sourced from the collection held at the Plant Tissue Culture Laboratory of the UCIARS. Ex-plants at the final multiplication stage (stage 7), and of similar height (0.5 cm) were selected for the experiment. They were then surface sterilized by immersion in pure ethanol for 30 seconds, followed by treatment with 10% sodium hypochlorite for 15 minutes. Residual disinfectant was eliminated by washing the explants three times with sterile distilled water. All root tissues were excised before transferring the explants to the prepared culture media. The cultures were monitored daily for growth and any signs of contamination.

2.6 Data collection and analysis

Number of roots, shoot height (mm), root dry weight (g), and shoot dry weight (g) were recorded. Collected data were subjected to analysis of variance using Minitab statistical software (version 17) and mean separation was performed using Tukey's test, at 5% significance level.

3. RESULTS AND DISCUSSION

3.1 Number of roots

The results presented in Table 2 indicate that the number of roots produced by the sour banana (*Mysore AAB*) plantlets varied significantly ($p<0.05$) among treatments from 1st to 5th week, while no significant difference ($p>0.05$) was observed during the 6th week. During the first week, T6 showed a marginally higher value, although not significantly different from the rest of the treatments except for T7. The early

development of roots is a key sign of how effectively explants adapt to *in-vitro* environments. Although the number of roots consistently increased between 2nd and 5th weeks, by the end of the 6th week, there was no significant difference ($p>0.05$) observed among the treatments, suggesting that AVG could be a suitable alternative to commercial IAA. Previous research conducted by Kumari et al. (2023) on the impact of *Aloe vera* gel extract (AVE) on the tissue culture of orchids grown on Knudson medium (KNC) have produced a higher plantlet height in *Aloe vera* gel treated media (9 KNC: 1 AVE treatment and 7 KNC: 3 AVE treatment) compared to the control (KNC only), emphasizing the possible advantages of AVE supplementation, especially in the early stages of orchid growth.

Table 2. Effect of different treatments on the number of roots of *Mysore AAB* banana

Treatment	Week 01	Week 02	Week 03	Week 04	Week 05	Week 06
T1	1.5 ^{ab}	3.6 ^{a}	4.9 ^{a}	5.9 ^{ab}	6.8 ^{ab}	7.3 ^{a}
T2	1.5 ^{ab}	3.7 ^{a}	4.8 ^{a}	5.1 ^{abc}	6.1 ^{ab}	6.8 ^{a}
T3	1.5 ^{a}	3.6 ^{a}	4.9 ^{a}	5.6 ^{abc}	6.4 ^{ab}	7.2 ^{a}
T4	1.0 ^{ab}	2.4 ^{ab}	3.5 ^{ab}	3.9 ^{bc}	4.4 ^{b}	4.8 ^{a}
T5	1.5 ^{a}	3.0 ^{a}	4.1 ^{ab}	4.7 ^{abc}	5.4 ^{ab}	6.3 ^{a}
T6	1.7 ^{a}	2.7 ^{ab}	4.5 ^{ab}	5.3 ^{abc}	5.5 ^{ab}	6.7 ^{a}
T7	0.7 ^{b}	1.6 ^{b}	2.7 ^{b}	3.5 ^{c}	4.4 ^{b}	4.8 ^{a}
T8	1.4 ^{ab}	3.3 ^{a}	4.8 ^{a}	6.3 ^{a}	7.1 ^{a}	7.5 ^{a}
T9	1.2 ^{ab}	2.7 ^{ab}	4.1 ^{ab}	5.3 ^{abc}	6.1 ^{ab}	6.5 ^{a}
Sig.	*	*	*	*	*	ns

'' represents significant difference and 'ns' represents non-significant difference. According to Tukey's Test, means in the same column followed by dissimilar letter/s in superscripts indicate significant difference at 0.05 level of probability.*

3.2 Shoot height (mm)

The results presented in Table 3 demonstrate that the plant height of sour banana plantlets showed statistically significant differences ($p<0.05$) among the treatments of this experiment. The plant height progressively increased in each treatment over the 6-week period. However, at the 6th week, except for T7, all the other treatments containing AVG showed comparable results to the control (T1) containing IAA. *Aloe vera* gel consists of natural growth hormones such as IAA, gibberellins, and salicylic acid that promote root initiation and elongation, making it a viable natural substitute to synthetic plant growth regulators (Vidanapathirana et al., 2023).

Table 3. Effect of different treatments on shoot height (mm) of *Mysore AAB* banana

Treatment	Week 01	Week 02	Week 03	Week 04	Week 05	Week 06
1	25.2 ^{a}	27.4 ^{ab}	32.1 ^{ab}	35.4 ^{ab}	39.3 ^{ab}	45.9 ^{ab}
2	24.3 ^{a}	26.8 ^{ab}	31.6 ^{ab}	36.4 ^{a}	44.6 ^{a}	51.5 ^{ab}
3	27.7 ^{a}	30.3 ^{a}	33.4 ^{a}	38.4 ^{a}	45.2 ^{a}	52.1 ^{a}
4	22.7 ^{ab}	25.2 ^{ab}	27.9 ^{ab}	30.6 ^{ab}	33.8 ^{ab}	39.6 ^{abc}
5	27.0 ^{a}	29.6 ^{a}	32.3 ^{a}	37.9 ^{a}	41.8 ^{a}	47.9 ^{ab}
6	28.2 ^{a}	31.4 ^{a}	33.7 ^{a}	41.2 ^{a}	46.2 ^{a}	52.3 ^{a}
7	14.8 ^{b}	18.3 ^{b}	20.8 ^{b}	23.1 ^{b}	24.9 ^{b}	27.1 ^{c}
8	23.2 ^{ab}	22.5 ^{ab}	25.6 ^{ab}	28.5 ^{ab}	31.8 ^{ab}	35.3 ^{bc}
9	25.0 ^{a}	28.5 ^{ab}	32.7 ^{a}	36.9 ^{a}	43.3 ^{a}	47.5 ^{ab}
Sig.	*	*	*	*	*	*

'' represents significant difference and 'ns' represents non-significant difference. According to Tukey's Test, means in the same column followed by dissimilar letter/s in superscripts indicate significant difference at 0.05 level of probability.*

3.3 Root dry weight (g)

The results presented in Figure 1 indicate that the dry weight of roots (g) of the sour banana plantlets, did not vary significantly among the treatments. This makes it clear that AVG provides sufficient growth-promoting substances to sustain root development and biomass accumulation, and, at the concentrations tested, can be utilized as an effective alternate for commercial auxins in producing tissue-cultured banana plants with similar root weight.

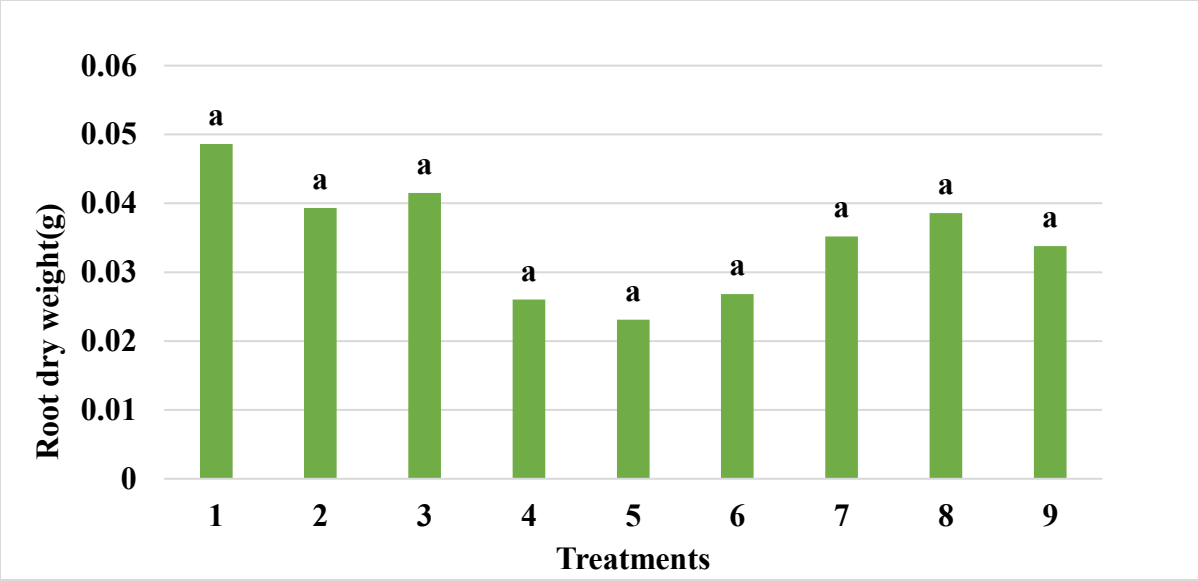

Fig. 1. Effect of different treatments on root dry weight (g) of *Mysore AAB* banana

According to Tukey's Test, bars with dissimilar letter/s indicate significant difference at 0.05 level of probability.

3.4 Shoot dry weight (g)

Significant differences were observed among the treatments ($p<0.05$) for the shoot dry weight of sour banana plantlets (Figure 2), with all AVG treatments, except T8, performing similar to the control (T1). A similar study conducted by Hamdeni et al. (2021) produced a higher shoot number in the culture media treatment containing *Aloe vera* gel (50 g/L *Aloe vera* gel + 0.2 mg/L IBA: β-indole butyric acid + 3mg/L BA: Benzyl amino-purine), compared to the control (0.2 mg/L IBA + 3mg/L BA + 0g/L *Aloe vera* gel). Even though the current study focused on shoot dry weight instead of the shoot number, the comparable biomass accumulation might partly be due to increased shoot growth and proliferation.

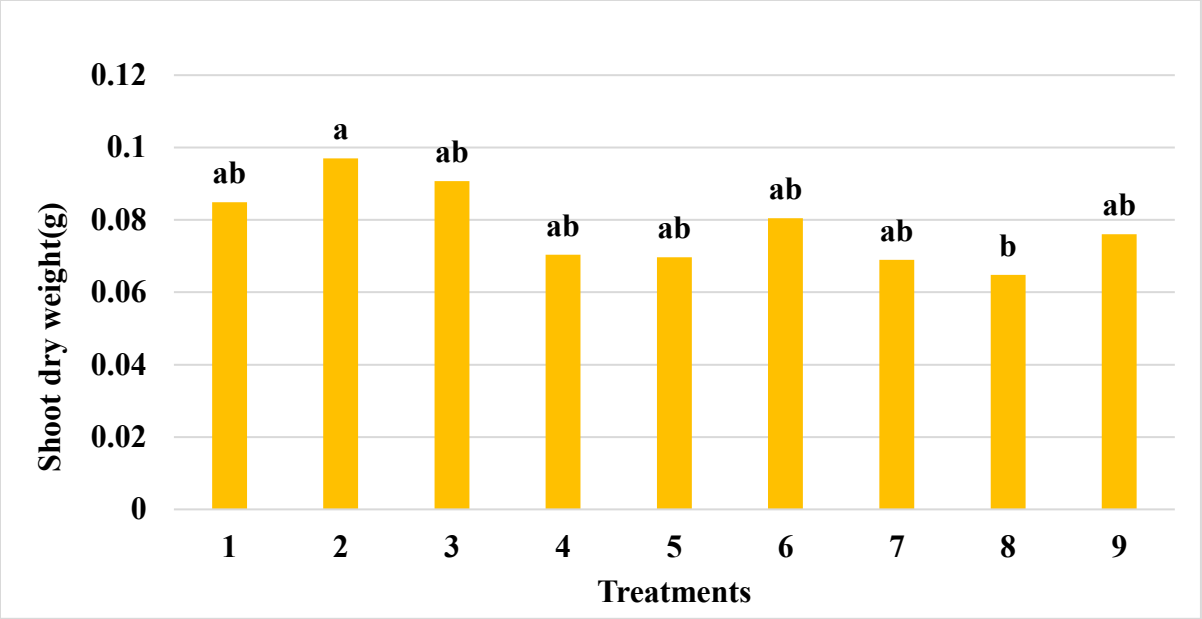

Fig. 2. Effect of different treatments on the root dry weight (g) of *Mysore AAB* banana

According to Tukey's Test, bars with dissimilar letter/s indicate significant difference at 0.05 level of probability.

4. CONCLUSION

The findings of this experiment show that *Aloe vera* gel (AVG) can effectively substitute commercial IAA in the tissue culturing of sour banana (*Mysore AAB*). Differences in root number observed during the early weeks disappeared by the 6th week, indicating similar rooting among the treatments. Most AVG concentrations also produced plant height, root dry weight, and shoot dry weight comparable to the control, with only a few concentrations showing reduced performance. Overall, AVG supports typical root and shoot development, validating its effectiveness as a substitute auxin source in tissue-cultured sour banana plantlet production.Further experimentation is needed to determine the exact amount of Aloe vera gel.

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