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Potential use of Aloe vera (*Aloe barbadensis* Miller) and Kessibissan (*Cyclea peltata* L.) as media additives for Anthurium tissue culture

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Abstract -Efforts to optimize protocols, explore alternative substances, and advance research are critical to making tissue culture a sustainable and cost-effective method for mass plant propagation. Hence, the present study was conducted at the Floriculture Research and Development Unit of the Royal Botanical Gardens, Peradeniya. The objective was to evaluate the potential of using Aloe vera (Aloe barbadensis Miller) and Kesibissan (Cyclea peltata L.) as nutrient additives and gelling medium for Anthurium tissue culture. The experiment was designed using a Complete Randomized Design with seven treatments, and each experiment was replicated 15 times. The number of leaves, shoots and roots per plantlet and the explant height were measured. Based on the results, our study found that supplementing Aloe vera and Kesibissan extracts had a substantial impact on the growth parameters of Anthurium explants after one month of incubation period. Kessibissan extracts performed well on Anthurium explant growth, especially in terms of the number of leaves, roots, and plantlet height. Meanwhile, the treatment involving 700 ml of MS media and 300 ml of Aloe vera extract proved to be the most successful for Anthurium, resulting in improved leaf, root, and shoot development. Overall, our findings give useful information for improving the growth of this attractive plant and may have applications for horticultural operations, highlighting the potential benefits of natural extracts in plant tissue culture.

Keywords: *Aloe barbadensis,* Anthurium, *Cyclea peltata*, Tissue culture, Gelling agents

I. INTRODUCTION

Micropropagation, also known as *in vitro* propagation or tissue culture, has transformed the floriculture industry by providing a highly effective way of mass-producing cut flowers (Chung *et al*., 2009). It allows for the rapid multiplication of superior cut flower species by carefully regulating environmental parameters such as light, temperature, and humidity and the exact delivery of growth hormones. This provides year-round availability of high-quality blooms, genetic homogeneity, and disease-free plantlets, making it a necessary tool for addressing the growing global demand for attractive and sustainable cut flowers (Suman, 2017). Due to the distinctive appearance of its inflorescence, the Anthurium (*Anthurium andraeanum* Lind.), a member of the Araceae family, is a known and valued flower. It is one of the most popular tropical flowers grown for commercial purposes (Pizano, 2005) and it ranks among the top ten species of cut flowers that are grown and traded globally (Satya and Maitra, 2007). Micropropagation technique has been frequently used for the mass production of these plants. Several recent studies have provided strong evidence that media additives facilitate germination, micropropagation and growth of different Anthurium cultivars (Maitra *et al*., 2012).

The availability of growth regulators and the nutritional ingredients supplied by the culture media are critical in influencing the growth and development of plantlets in tissue culture (Espinosa-Leal *et al*., 2018). Nowadays, the cost of some chemicals needed for plant tissue culture, particularly for agar and growth regulators is rising quickly. Researchers and business professionals have been investigating alternative ways and chemicals in response to the escalating costs of growth regulators and agar (Vanisree *et al*., 2004; Rathnayaka et al., 2023b). This involves the creation of low-cost formulations, the use of additional gelling agents such as gellan gum, and the research of naturally produced growth regulators such as coconut water, tomato juice, peptone, and extracts from potato, banana and beef (Asha Amal, 2016). These initiatives seek to lessen the financial burden of tissue culture while retaining its effectiveness.

In this regard, Aloe vera gel was used as an organic nutritional supplement to improve the *in vitro* growth process of plants in a previous study (Hamdeni *et al*., 2022). Because of its biological effectiveness and chemical makeup (carbohydrates, organic acids, proteins, phenolic compounds, vitamins, minerals, and amino acids), the Aloe vera gel is the most often used component of the plant (Flores-Lopez *et al*., 2016). Furthermore, the secondary metabolites and antioxidant profile of Aloe vera gel reveal the plant's immense potential for medical, pharmacological, and aesthetic uses (Cardarelli *et al*., 2017). The leaves of Kesibissan, also known as Kahipittan in Sri Lanka, are used to make delectable herbal jelly. Before the invention of the contemporary jelly, filled with artificial substances and chemicals, Sri Lankans had been consuming it. This jelly's unique quality is its ability to set at ambient temperature. Therefore, to make this natural and herbal jelly, there is no need for a refrigerator. This jelly is still prepared and consumed as a treat by people in remote parts of Sri Lanka (Siriwardhana *et al*., 2018). Hence, this study aimed to evaluate the potential of using Aloe vera (*Aloe barbadensis* Miller*)* and Kesibissan (*Cyclea peltata* L.) as nutrient additives and gelling medium for Anthurium cultures.

II. MATERIALS AND METHOD

A. Study area

The experiment was carried out in the tissue culture laboratory facilities of the Floriculture Research and Development Unit at the Royal Botanical Gardens, Peradeniya (7° 16' N, 80° 35' E), which is located in the agro-ecological zone (WM 3a) of Sri Lanka.

B. Planting materials

The young Anthurium (Lj21 (Lalani)) cultivar plantlets (each 1 cm in height) were taken from the existing collection of explants from the Royal Botanical Gardens. The explants were immersed in a 10 % Sodium Hypochlorite solution for 5 – 10 minutes and washed thrice using autoclaved distilled water for surface sterilization before incubation (Pradhan *et al*., 2013). The kesibissan leaves were cleaned thoroughly using 70 % Alcohol and then washed using distilled water. Leaves (30 g) were ground using a blender consisting of 300 ml of distilled water and filtered using double-layered muslin cloth to obtain the leaf extracts separately. Meanwhile, Aloe vera leaves were cleaned using 70 % alcohol and then washed with detergent (Teepol). The thornson sides of Aloe vera leaves were removed and the leaves were ground using a blender without water.

C. Media preparation and culture conditions

Different strengths of MS (Murashige and Skoog) media were prepared for Anthurium cultures as shown in Table 1. After that 4 % (W/V) of sugar and 1.27 % (W/V) of agar were added and the pH of the media was adjusted to 5.60 - 5.63. The media were then autoclaved at 120 °C for 15 minutes and 40 ml of each medium was poured into sterilized culture bottles (100 ml). Then the surface sterilized Anthurium explants were established into culture bottles inside a laminar flow with one culture vial holding four small plantlets. Cellophane layers were used to seal the culture bottles, which were then kept in a growth room at 25 °C and 16 hours of photoperiod under fluorescent lighting (40 µmol photons $m^{-2}s^{-1}$).

D. Data collection

The following data were recorded after one month of Anthurium explant inoculation. The number of leaves, shoots, and roots, as well as shoot length, were measured using 1mm graph paper.

E. Data analysis

The treatments were arranged in a CRD (Completely Randomized block Design) method having 15 replicates where each replicate consisted of 4 plantlets. The data

Table 1**:** Different media for Anthurium culture

Treatment	Media combination
T1	900ml of MS + 100ml of Aloevera extract
T2	800 ml of MS+ 200 ml of Aloevera extract
T3	700 ml of MS+ 300ml of Aloevera extract
T4	980ml of MS + 5g of Agar + 20 ml of Kesibissan extract
T5	950 ml of MS+ 5g of Agar + 50 ml of Kesibissan extract
T6	900 ml of MS+ 5g of Agar + 100 ml of Kesibissan extract
T7 (Control)	1litre of MS media

The data obtained were distributed normally and evaluated using the SPSS software and analysis of variance (ANOVA) was performed to check if any treatments differ significantly at Tukey's 5 % level of probability.

III. RESULTS AND DISCUSSION

A. Effect of different media on number of leaves per plantlet

Different treatments had a significant effect ($p = 0.001$, $F = 11.661$ $df = 6$)) on a number of leaves per explant of Anthurium after one month of incubation period. The highest number of leaves were recorded in T3 (8.8 ± 0.3), T4 (8.6 ± 0.3), T5 (8.6 ± 0.3) and T6 (8.6 ± 0.3) compared to other treatments (Figure 1).

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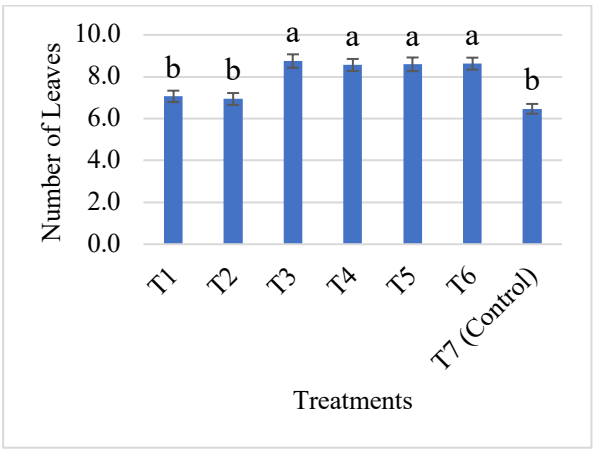

Figure 1: Effect of different media on the number of leaves per Anthurium plantlet. Error bars with different letters are significantly different at Tukey's 5 % level of probability, p = 0.001, F = 11.661 df = 6, n = 15 (number of replicates in each treatment)

B. Effect of different media on number of shoots per plantlet

Significant differences ((p = 0.001, F = 24.505 df = 6)) were observed in number of shoots per explant. Treatment T3 (2.3 ± 0.1) had the highest number of shoots followed by T2 (1.7 ± 0.1) and T1 (1.6 ± 0.1) while T4 (1.0 ± 0.0) and T7 (1.0 ± 0.0) had the lowest. Anthurium explant grown with Aloe vera extracts produced a higher number of shoots per plant compared with control and Kessibissan extracts (Figure 2).

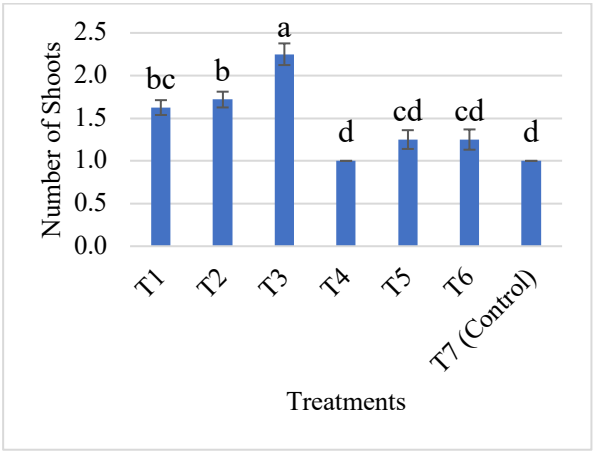

Figure 2: Effect of different media on the number of shoots per Anthurium plantlet. Error bars with different letters are significantly different at Tukey's 5 % level of probability, p = 0.001, F = 24.505 and df = 6, n = 15 (number of replicates in each treatment)

C. Effect of different media on number of roots per plantlet

Number of roots per plantlet was significantly affected ((p = 0.001, F = 23.828 df = 6)) by different media combinations. The highest number of roots per plantlet was observed in T3 (6.3 ± 0.2) followed by T6 (5.4 ± 0.3) and T5 (5.0 ± 0.3) while the lowest was in T1 (3.3 ± 0.2) and T7 (2.9 ± 0.3) (Figure 3).

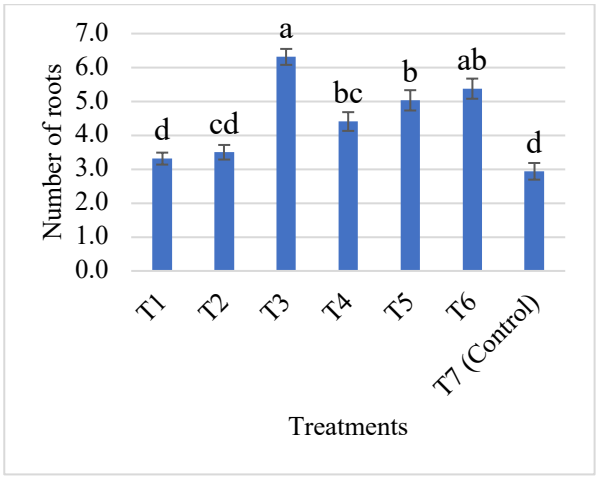

Figure 3: Effect of different media on the number of roots per Anthurium plantlet. Error bars with different letters are significantly different at Tukey's 5 % level of probability, p = 0.001, F = 23.828 and df = 6), n = 15 (number of replicates in each treatment)

D. Effect of different media on plantlet height

Different treatments had a significant effect ((p = 0.001, F = 32.167 df = 6)) on the explant height of Anthurium after one month of the incubation period. The highest plant height was recorded in T6 (4.48 ±0.1) followed by T5 (4.07 ± 0.2) and T4 (3.87 ± 0.2) while the lowest was in T7 (2.31 ± 0.1). Anthurium explant grown with Kessibissan extracts produced taller plants compared with control and Aloe vera extracts (Figure 4).

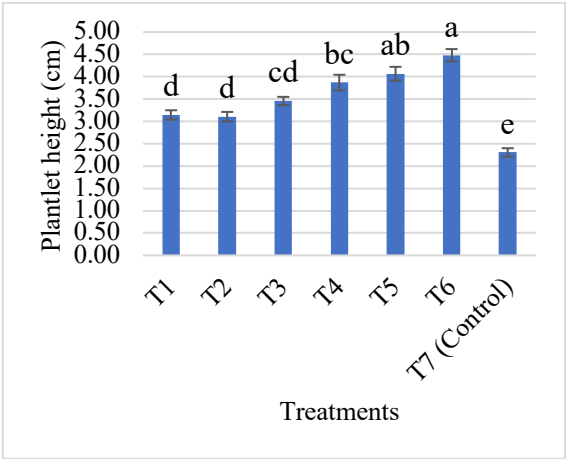

Figure 4: Effect of different media on height of Anthurium plantlet. Error bars with different letters are significantly different at Tukey's 5 % level of probability, p = 0.001, F = 32.167 and df = 6), n = 15 (number of replicates in each treatment)

Finally, the findings of this study show that using Aloe vera and Kessibissan extracts significantly altered the growth characteristics of Anthurium explants after one month of

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incubation. However, Kessibissan extracts had a substantial impact on Anthurium explant growth, notably in terms of the number of leaves, roots, and plantlet height. Meanwhile, Aloe vera extract resulted higher number of shoots compared with control and Kessibissan extracts. Several previous studies have proven the success of incorporating organic additives in the tissue culture media of Anthurium. Rathnayake *et al*. (2023a) concluded that 1/4 MS+15 g/l neem leaf powder increased the number of leaves per plantlet (7.25) of Anthurium explant while Full MS+5 g/l neem leaf powder raised the number of roots per plantlet (2.88). The treatments with 1/2 MS+10 g/l moringa leaf powder (1.56), 1/4 MS+15 g/l moringa leaf powder (1.69), and 1/2 MS+10 g/l neem leaf powder (1.69) produced considerably more shoots per plantlet than the control treatment (1.06). On the other hand, all three concentrations of neem leaf powder boosted Anthurium explant shoot length more than other treatments. Maitra *et al*. (2012) investigated the direct regeneration of Anthurium from adventitious bud explant. The most successful medium in terms of explant survival (84.45 %), earliest shoot emergence, and number of shoot and leaf production per culture at the time of initial transfer was half-MS media supplemented with coconut water 15% (v/v), NAA (1 mg l^{-1}), and BAP (3 mg l^{-1}).

IV. CONCLUSIONS

We investigated the utilization of Aloe vera and Kesibissan extracts in Anthurium tissue culture in our study at the Royal Botanical Gardens. Results demonstrated that both Aloe vera and Kessibissan extracts could be useful supplements for promoting Anthurium plant growth. Whereas Kesibissan significantly impacted plantlet height, and leaf and root development, Aloe extract resulted higher number of shoots compared with the rest of the treatments. Meanwhile, 700 ml of MS + 300 ml of Aloe vera extract significantly impacted the overall Anthurium growth beneficially. The potential of natural extracts in tissue culture and practical insights into how to improve plant growth are provided by these discoveries. Optimizing extract concentrations, carrying out longer-term trials, and investigating molecular pathways should be the main goals of future research in this field. Field experiments, varietal-specific research, and environmental evaluations can also offer useful information, and cost-benefit studies should examine the economic viability. Aloe vera and Kessibissan extracts can be used in tissue culture for sustainable and affordable plant propagation in horticulture. This use can be improved by examining disease resistance, scaling up production, and interdisciplinary cooperation.

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