Aloe Vera (*Aloe barbadensis* miller) Extract as Media Supplement in Orchid Tissue Culture

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Abstract

The use of natural substances as media supplements to promote the growth and development of orchid has received a lot of interest. Aloe vera extract which is well-known for its nutritional characteristics, has the potential to influence the in vitro growth of orchid plants. Hence, the present study was conducted in the Floriculture Research and Development Unit at the Royal Botanical Gardens, Peradeniya to evaluate the potential of usage Aloe vera extract as nutrient additive for Orchid tissue culture. The experiment was arranged in Complete Randomized Design having four treatments with 15 replicates for each experiment. The number of leaves, shoots and roots per plantlet and explant height were measured. The results showed that various treatments had a substantial impact on the growth characteristics of Orchid explants. During the third week of incubation, 900ml of KNC+100ml of Aloe vera extract and 700ml of KNC+300ml of Aloe vera extract, consistently promoted higher numbers of leaves and shoots and plantlet height compared to the control (KNC media), highlighting the potential benefits of Aloe vera extract supplementation, particularly in the early growth stages. These benefits continue throughout the fifth week of incubation, with all treatments except the control. Notably, 800ml of KNC+200ml of Aloe vera extract produced the most shoots per explant. As a result, these findings emphasize the potential benefits of including Aloe vera extract in orchid tissue culture media, particularly during the early phases of growth.

Keywords: Aloe Vera Extract, In Vitro Growth, Media Supplements, Orchid

I. INTRODUCTION

Because of their astonishing beauty and diversity, orchids are among the most valuable ornamental plants in the world (Chugh et al., 2009). However, numerous obstacles stand in the way of their cultivation and propagation due to their slow growth rates and susceptibility to diseases. Orchids reproduce naturally through seeds, but in the absence of suitable hosts, the seeds do not germinate in sufficient numbers. These challenges can be overcome by using tissue culture techniques. The micro propagation technique, the most often utilized biotechnological tool for producing ornamental plants commercially is very fast and effective compared to the conventional methods.

All the nutrients necessary for a plant's regular growth and development are present in plant tissue culture media, such as macronutrients, micronutrients, vitamins, amino acids, sugar, other nitrogen and organic supplements, growth regulators, and solidifying agents (Hussain et al., 2012). In general, orchid tissue culture media contains water, vitamins, and mineral salts and as organic additives in orchid growth medium, coconut water, tomato juice, peptone, potato, banana, and beef extracts are commonly employed. A number of recent research have found that medium additives aid in the germination, micropropagation, and growth of many orchid varieties (Rathnayaka et al., 2023b; Tawaro et al., 2008) and other ornamental crops (Rathnayaka et al., 2023a; Maitra et al., 2012).

In this regards, *Aloe vera* gel has been employed in a prior study as an organic nutritional supplement to enhance the growth of plants *in vitro* (Hamdeni et al., 2022). Aloe vera gel is the part of the plant that is employed the most frequently due to its biological efficacy and chemical composition (carbohydrates, organic acids, proteins, phenolic compounds, vitamins, minerals, and amino acids). Furthermore, the plant's enormous potential for therapeutic, pharmacological, and aesthetic uses is revealed by the secondary metabolites and antioxydant profile of Aloe vera gel (Cardarelli et al., 2017). Hence, the objective of this study is to evaluate the

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impacts of Aloe vera extract as a media supplement in orchid tissue culture. Understanding the complicated interactions between Aloe vera extract and orchid tissue culture media will pave the way for more effective and sustainable orchid production, helping to conserve these extraordinary plants and the thriving ornamental sector.

II. MATERIALS AND METHODS

A. Study Area

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

B. Planting Materials

The young *Dendrobium* orchid plantlets (each 1 cm height) were taken from the existing collection of explants from 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 (Ranthnayaka et al., 2023b). Aloe vera leaves were cleaned using 70 % alcohol and then washed by "Teepol". The thorns in the both side 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 KnC (Knudson) media were prepared for Orchid cultures separately as shown in Table 01. 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 Orchid explants were established into culture bottles inside a laminar flow with one culture vial held 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 in Orchid cultures at 3, 5 and 7 weeks after explant inoculation. The number of leaves, shoots and roots per plantlet were counted. Shoot length was measured using 1 mm graph paper.

Table 01: Different Media for Orchid Cultures using Aloe Vera Extract

Treatment	Media combination
T1 (Control)	1litre of KNC media
T2	900ml of KNC + 100ml of Aloe vera extract
T3	800 ml of KNC+ 200 ml of Aloe vera extract
T4	700 ml of KNC + 300ml of Aloe vera extract

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 obtained were distributed normally and evaluated using the SPSS software. Additionally analysis of variance (ANOVA) was performed to check whether there were treatments having differed significantly at Tukey's 5 % level of probability.

III. RESULTS AND DISCUSSION

A. Effect of Aloe Vera Extract on Number of Leaves of Orchid Plantlets

The number of leaves per Orchid explant was significantly affected ($p<0.05$) by different treatments during 3rd and 5th week after incubation. During 3rd week after incubation, T2 (900ml of KNC + 100ml of Aloevera extract) (4.8) and T4 (900ml of KNC + 100ml of Aloe vera extract) (4.6) produced significantly higher number of leaves per explant while the control T1 (KNC media) produced the lowest (2.8). During 5th week, all the treatments except T1 (4.2) had higher number of leaves (5 – 5.2). However, during 7th week after incubation, no significant differences were observed the number of leaves of Orchid explant ($p>0.05$) (Figure 01).

B. Effect of Aloe vera Extract on Number of Shoots of Orchid Plantlets

Different treatments had significant effect ($p<0.05$) on the number of shoots per Orchid explant during 3rd and 5th week after incubation. During 3rd week after incubation, all the treatments except control (T1 = 1) produced higher number of shoots per explant (T2 = 1.8, T3 = 2.2 and T4 = 2.1). The treatment T3 resulted the highest number of shoots per explant (2.9) during

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5th week after incubation while T1 resulted the lowest (1.7) (Figure 02).

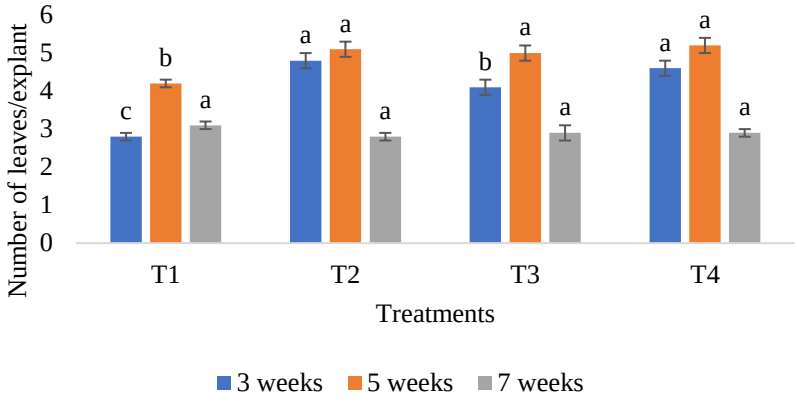

Figure 01: Variations in the Number of Leaves of Orchid Plantlets Grown with Aloe Vera Extracts. Bars with different letters represent significant differences at Tukey's p<0.05. CV – Coefficient of Variance, n=15

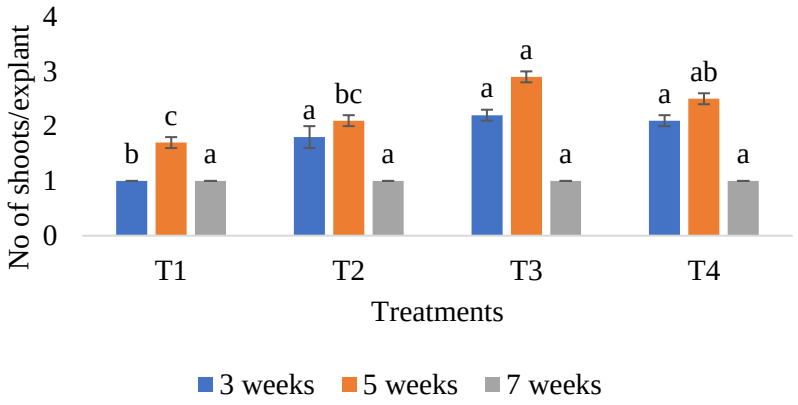

Figure 02: Variations in the Number of Shoots of Orchid Plantlets Grown with Aloe Vera Extracts. Bars with different letters represent significant differences at Tukey's p<0.05. CV – Coefficient of Variance. N=15

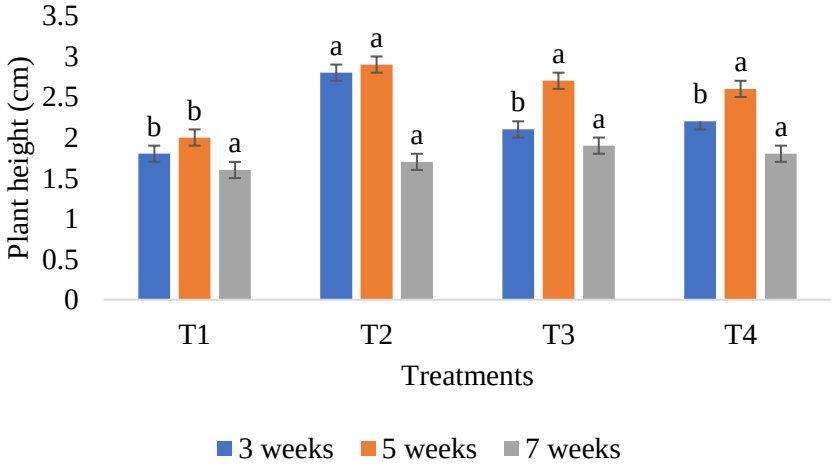

Figure 03: Variations in the Plant Height (cm) of Orchid Plantlets Grown with *Aloe vera* Extracts. Bars with different letters represent significant differences at Tukey's p<0.05. CV – Coefficient of Variance. N=15

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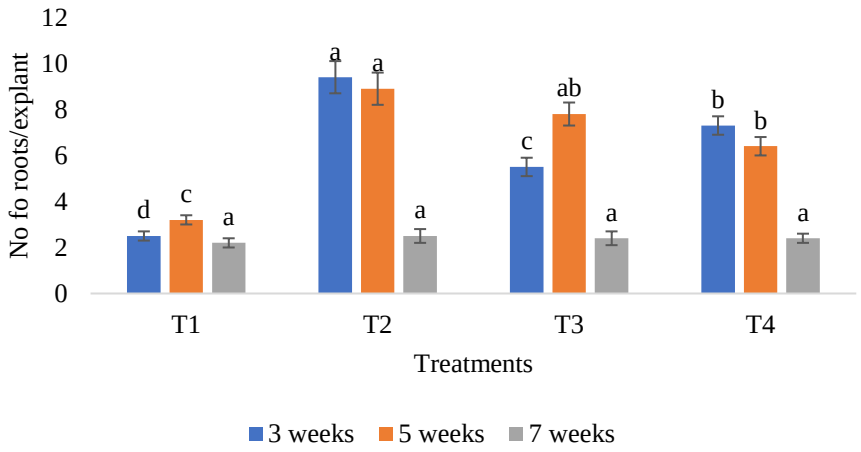

Figure 04: The number of roots of Orchid plantlets grown with Aloe vera extracts. *Bars with different letters represent significant differences at Tukey's p<0.05.* CV – Coefficient of Variance. N=15

C. Effect of Aloe vera Extract on Plant Height of Orchid Plantlets

The plant height of Orchid explant was significantly affected ($p<0.05$) by different treatments during 3rd and 5th week after incubation. During 3rd week after incubation, the highest plantlet height (2.8 cm) was observed in T2 (900ml of KNC + 100ml of Aloe vera extract) compared with other treatments. During 5th week, higher plantlet heights were observed in all the treatments except T1 (Figure 03).

D. Effect of Aloe Vera Extract on Number of Roots of Orchid Plantlets

Different treatments had significant effect ($p<0.05$) on the number of roots per Orchid explant during 3rd and 5th week after incubation. The highest number of roots were in T2 during 3rd (9.4) and 5th (8.9) week after incubation respectively. The T1 treatment resulted the lowest number of roots (2.5 and 3.2 respectively) (Figure 04).

The findings of this study show that different treatments have a substantial impact on the growth characteristics of Orchid explants at various phases of incubation. Treatments containing a combination of KNC media and Aloe vera extract, such as T2 and T4, consistently promoted the maximum number of leaves, shoots, and plantlet height during the third week after incubation as compared to the control treatment (T1). These findings highlight the potential benefits of supplementing Orchid tissue culture media with Aloe vera extract, particularly during the early phases of growth. The favourable influence of the treatments was maintained as the experiment advanced to the fifth week following incubation. Except for the control, all treatments continued to produce more leaves and shoots, with T3 producing the most shoots per explant. Furthermore, during this time span, treatment T2 consistently produced the tallest plantlets, confirming the beneficial effect of Aloe vera extract on the Orchid.

IV. CONCLUSION

Several previous studies have proven the success of incorporating organic additives in tissue culture media of Orchid. Rathnayaka et al. (2023b) evaluated the potential usage of Moringa and neem additives in Orchid tissue culture. Based on their results, Moringa and neem leaf extracts both performed better than moringa powder. 14 KnC+15g/l moringa leaf extract produced an increased number of leaves and roots per plantlet, whereas full KnC+5g/l moringa leaf extract produced the maximum number of shoots per plantlet (3.5). On the contrary, neem leaf extracts (5g/l, 10g/l, and 15g/l) boosted orchid explant shoot length and produced a much higher number of roots per plantlet. As a result, moringa and neem leaf extracts can be employed to create a simple and low-cost culture media for supporting orchid tissue culture. According to Aktar et al. (2008), in laboratory circumstances, the interaction of various media and organic additives had a substantial effect on the growth and development of protocorm like bodies and subsequent plantlets regeneration of *Dendrobium* orchid. Except for shoot and leaf length, the

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interaction of ½ MS medium with Sabri banana pulp yielded the highest values for all metrics. The longest shoots, however, were discovered in KNC medium with Sabri banana pulp, and the longest leaves were discovered in both KNC and 1/2MS media with Sabri banana pulp.

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