Research Article

A Comparative Study of Anthurium Differentiation by Direct and Indirect Methods Using the Plant Tissue Culture Technique

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Abstract | Anthurium is a commercially widespread ornamental flower genus with a vast species variation. A low-cost and relatively fast method for anthurium mass production is of high significance. Hence, the study aimed to evaluate the efficacy of using growth regulators and (BA) Benzyladenine for accelerating anthurium callus growth and differentiation in tissue culture technique. The method included planting 1 cm long pieces of Anthurium leaf on MS medium supplied with growth regulators 2,4-D at 0.0, 0.25, 0.50, or 0.75 mg.L^{-1} with BA at 0.0, 0.5, 1.0, 1.5, or 2.0 mg.L^{-1} during the callus induction stage. Then, plant propagation using half-dose MS medium supplied with NAA at 0.0, 0.1, or 0.5 with BA at 0.0, 1.0, 2.0, or 3.0 mg. L^{-1} was compared between the indirect method, when using 100 mg callus, and the direct method by producing plants from anthurium leaf pieces. The results showed that the best callus production was 1.25 g when using 2,4-D at 1.5 in combination with 0.75 BA medium treatment. Findings also showed that plant production by the indirect method using leaf callus could produce 32.2 plants on medium treated with 2 mg. L^{-1} BA with 0.1 mg. L^{-1} NAA, while the direct method of leaf culture produced fewer plants, not exceeding 18.6 plants at concentrations of 2.0 and 0.1 mg. L^{-1} of NAA and BA, respectively. The data clearly show that the best treatment for callus induction in Anthurium leaf explants is with MS medium containing a combination of 2.0 mg L^{-1} BA and 0.75 mg L^{-1} 2,4-D. This combination produced an excellent quality callus as well as quantity compared to other concentrations tested, thus confirming that there is a synergistic effect between BA and 2,4-D on cell proliferation and differentiation by tissue culture process.

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Introduction

Anthurium is the largest genus in the family Araceae, constituting more than 800 species (Reimuth and Zotz, 2020). The neotropical genus Anthurium is the largest and most complex genus, which includes more than 100 species. Anthuriums are commercially produced as ornamentals for their showy and colourful spadix and are widely used for cut flowers as well as potted garden plants. They are semi-tropical, perennial, and epiphytic plants, native to South-West Columbia, which was brought to

Europe in 1876 (Singh, 1987).

The Netherlands is the world's leading producer of anthuriums, growing 25 thousand stems a year, followed by Hawaii and Mauritius that which produce 11 and 10.2 thousand, respectively (Ortíz, 2008). In Mexico, anthuriums are grown in approximately 20 ha, distributed in the states of Veracruz, Chiapas, and Mexico (Gallaga, 2000). Anthurium andreanum and Anthurium scherzerianum are the most cultivated species in Mexico and in the world (Gantait and Mandal, 2010). A. andreanum is the most important species from an economic standpoint, since it has the largest number of commercial varieties (López-Puc *et al*., 2013)

Seed propagation of Anthurium leads to genetic divergence due to high heterozygosity, affecting quality, yield, and time to first flowering in commercial plantations (Jahan *et al*., 2009). Additionally, seeds exhibit short-term viability (2-3 days after harvest) and a low germination rate (20-30%) (Jahan *et al*., 2009; Gantait *et al*., 2012). Due to the slow vegetative development of Anthurium, asexual propagation through the shoot or stem part is time-consuming (Cardoso and Habermann, 2014; Alwan *et al*., 2025; Talib *et al*., 2025). For the above reasons, it is necessary to develop a clonal propagation method for large numbers of commercial propagations in a short time (Cardoso and Habermann, 2014). In order to meet the demand for Anthurium, which is an indoor plant, biotechnological methods are used in addition to classical production methods. Biotechnological methods, especially plant tissue cultures and molecular techniques, come to the forefront in order to support growers and producers in meeting the demands of the next century in the ornamental plants sector. Moreover, micropropagation, being a useful alternative for the propagation of Anthurium, is a rapid method suitable for application in small areas to produce pathogen-free plants (Martínez-Estrada *et al*., 2016). This study aimed to produce anthurium plants from leaf cut callus with the aid of growth regulators BA and 2,4-D, and to compare indirectly produced plants from callus with the directly produced from the leaf using the growth regulators BA and NAA.

Materials and Methods

Preparation of plant materials

Anthurium seedlings were collected from nurseries at or before the flowering stage and kept in the greenhouse to obtain plant parts of the anther, petal, leaf, and young leaves. The plant parts: young leaves, petals, and anthers were taken and placed in a 250ml flask, and carefully washed with tap water and liquid soap. Then, they were sterilised using the laminar air flow cabinet. Sodium hypochlorite (NaOCl) was added to them to sterilise the plant parts at concentrations of (0, 1, 2, and 3) % for different time periods at (5, 10, 15, and 20) minutes. Then, the plant parts were transferred to A 250ml glass container containing 70% ethanol for one minute, washed with distilled water three times, to be ready for the experiment procedure.

Medium preparation

For preparing one litre of plant tissue culture medium, an amount of 4.49g of ready-mixed culture medium powder was added to 7 g agar (solidifying), 3% sucrose and BA, 2,4-D, and NAA according to each corresponding treatment. The medium was reduced to 5.6 by adding sodium hydroxide solution (NaOH). The medium was placed on a hot magnetic stirrer and then poured into test tubes at a rate of 10 ml per tube. After closing them tightly, they were placed in an autoclave at a temperature of 121°C and a pressure of 1.04 kg/g cm2 for 15 minutes. Then, they were removed from the autoclave and left to cool until the medium solidified at room temperature, thus becoming ready for cultivation. l

Experiment procedure

For callus production, the plant parts were then placed in Petri dishes, cultured medium treated with 2,4-D at concentrations of 0.0, 0.25, 0.50, 0.75 mg $L^{-1,}$ and cytokinin BA at concentrations of (0.0, 0.5, 1.0, 1.5, 2.0). At the callus induction stage, the culture media containing half-dose MS medium were treated with NAA at 0.0, 0.1, 0.5, and BA at 0.0, 1.0, 2.0, and 3.0 mg L^{-1}. The samples were incubated in a growth chamber at 25∓°C and 1000 lux light intensity for 16 hours, followed by 8 hours of darkness. The effects of growth regulators were compared on plant production from callus by the indirect method against plant production by the direct method using plant leaves.

Experimental design and data analysis

The experimental treatments included all possible interactions, which were distributed as a Randomised Complete Block Design (RCBD) with three

replications. The experiment data were collected and subjected to data analysis, and analysis of variance ANOVA was performed with the aid of GenStat 12th (14). Differences among the treatment means were compared according to the least significant difference (LSD) at a probability level of 0.05.

Results and Discussion

Determination of 2,4-D and BA best concentration for callus production

The results (Table 1) showed that the 2,4-D and BA concentrations differed in their effect in increasing the fresh weight rate of callus induced from the 1 cm^2 Anthurium leaf 45 days post-cultivation, as the used concentrations of BA significantly outperformed the comparison treatment. The BA at 2.0 mg L^{-1} gave the highest fresh weight rate of 1.03 g compared to the lowest fresh weight of 0.07 g produced in the control. Similarly, the 0.75 mg L^{-1} of 2,4-D significantly outperformed the other concentrations and resulted in callus fresh weight of 0.82 g compared to its negative control that gave only 0.45 g.1The results also indicated a significant superiority of the interaction between BA and 2,4-D in increasing induced callus fresh weight. The combination of BA at 1.5 mg L^{-1} and 2,4-D at 0.75 mg L^{-1} gave the highest callus fresh weight of 1.25 g among all the treatments.

Table 1: *Callus fresh weight (g) yielded from a 1 cm^2 leaf on MS medium treated with 2,4-D and Benzyl Adenine (BA) after 45 days of cultivation*

Treatments	2,4-D (mg L^{-1})				Average
BA (mg L^{-1})	0.0	0.25	0.50	0.75	
0	0	0	0.13	0.15	0.07
0.5	0.21	0.18	0.22	0.34	0.24
1.0	0.57	0.75	0.79	1.21	0.83
1.5	0.67	0.98	1.13	1.25	1.01
2.0	0.82	0.92	1.22	1.15	1.03
Average	0.45	0.57	0.70	0.82	
LSD p≤0.01	BA= 0.15 2,4-D= 0.11 Interaction= 0.246				

The growth weight of fresh callus was increased by treating the medium with benzyl adenine. This indicates that BA seems to have a role in balancing the positive and negative charges on both sides of the cell membrane and thus increases the absorption of other growth regulators. Such activities lead to an increase in the level of vital construction, including increased protein construction and cell division, and finally an increase in fresh weight (Dodds and Robert, 1985). According to (Jarmt 2023), there.

There were significant differences in the interaction between the growth regulators 2,4-D and BA in increasing the percentage of callus induction. This is mostly due to the auxin effect, which stimulates cell wall flexibility by breaking the cell wall bonds and relocating them under turgor pressure, which increases the cell size and expansion (Taiz and Ziger, 2010).

Table 2: *Number of differentiated plants from approx. 100 mg callus FW on MS medium treated with NAA and BA mg L-1 after 45 days post-cultivation*

Treatments	NAA (mg L^{-1})			Average
BA (mg L^{-1})	0.0	0.1	0.5	
0	3.6	6.7	5.9	5.4
1	12.2	23.3	13.5	16.33
2	25.2	32.3	23.2	27.23
3	14.4	13.4	14.2	14.00
Average	14.10	18.93	14.2	
LSD p≤0.01	BA= 2.51 NAA= 3.23 Interaction= 5.34			

The treatment's effect on plant production by the indirect method

The MS medium treated with BA showed an increased number of plants from the vegetative Anthurium callus after 45 days of cultivation compared to the untreated medium (Table 2). The highest number of 27.23 plants was grown using BA at a concentration of 2.0 mg L^{-1}, which differed significantly from the other concentrations, while the control medium produced only 5.4 plants. It was also shown (Table 2) that individual NAA concentrations differed in their effect on the number of plants produced from the Anthurium callus. Medium treated with 0.1 mg L^{-1} NAA was superior to the other concentrations, giving the highest number of differentiated plants from fresh callus (18.93 plants) compared to only 14.4 plants from the untreated control culture medium. Among all the treatments, the interaction of 2.0 BA and 0.1 NAA resulted in the highest plant production number that reached 32.3 plants with significant differences from all the other individual and interaction treatments (Figure 1C).1

Kour and Singh (2012) have indicated the importance of adding cytokinin to the nutrient medium during the multiplication stage. This can assist plant parts to

grow from a vegetative branch by balancing with the internal plant auxins and working to form the lateral branches and better growth.

Treatments' effect on plant production by the direct method

The results shown in Table 3 indicate that BA at a concentration of 2.0 mg L^{-1} increased the average number of plants from the leaf to 14.4 differentiated plants, while no plants were grown from the control treatment. It can be noticed from the same table, there was a significant effect of the growth regulator NAA on the number of plants, as the 0.1 mg L^{-1} NAA was superior in increasing the number of differentiated plants to 10.4 plants, which was superior to the control treatment that reached only 4.78 plants. The combined BA with NAA always resulted in an increase in the number of differentiated plants directly from a 1 cm^2 leaf piece after 45 days of planting (Figure 1. A, B, C, and D). Interaction of 2.0 mg L^{-1} BA and 0.1 mg L^{-1} NAA gave the highest number of 18.6 plants. l

Table 3: *Number of differentiated plants from 1cm² anthurium leaf cut directly grown on MS medium treated with NAA and BA mg L^{-1}, 45 days post-cultivation*

Treatments	NAA (mg L^{-1})			Average
BA (mg L^{-1})	0.0	0.1	0.5	
0	0	0	0	0
1	4.2	15.3	10.5	10.00
2	9.4	18.6	15.2	14.4
3	5.5	7.7	8.6	7.27
Average	4.78	10.4	8.58	
LSD p≤0.01	BA= 3.21 NAA= 3.54 Interaction= 5.32			

Khaleghi *et al.* (2008) have confirmed that adding low concentrations of auxin NAA with a lower percentage than cytokinin BAP increases the growth rate and multiplication compared to using cytokinin BAP only. The reason for the increased branching rate when adding BA to the nutrient medium may be due to its role in abolishing apical dominance and liberating lateral buds, thus increasing the number of vegetative branches. The effectiveness of BA in branch multiplication is attributed to the side chain containing three double bonds (Bruyn, 1992). Auxin NAA prevents vascular communication between the vascular tissues of the axillary buds and the vascular tissues of the stem, which leads to the lack or lack of passage of nutrients from the stem tissues to the buds, and thus their growth is weak (Kim *et al.*, 1991).

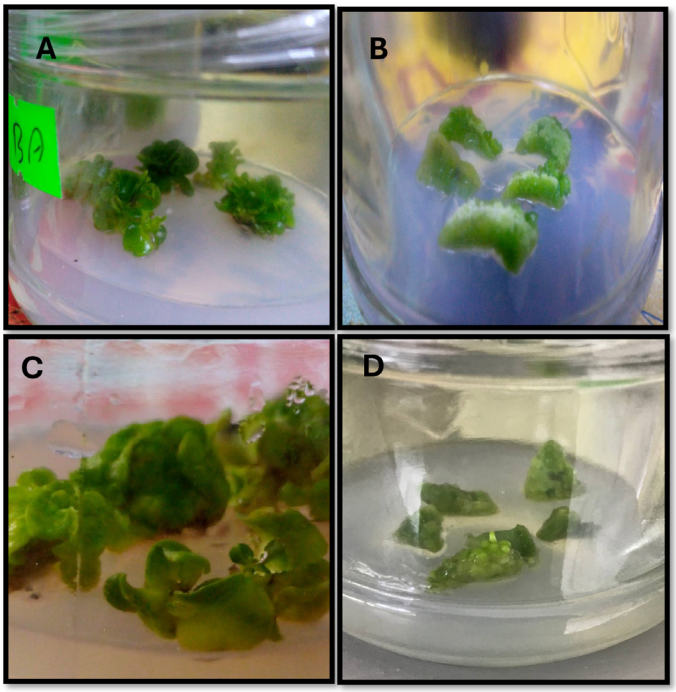

Figure 1: *A: Callus production from the leafy part of the plant; B and D: plant differentiation directly from the leaf; C: Plant differentiation from callus formed on the leafy plant part*

Conclusions

The research showed the effect of growth regulators (BA and 2,4-D) added to MS medium in stimulating callus production from Anthurium leaves, where the best concentrations used in callus induction were 2.0 mg L^{-1} of BA and 0.75 mg L^{-1} of 2,4-D, and the possibility of a specific combination of producing plants from callus using growth regulators BA and NAA, The research also showed the production of plants directly from the leaf using growth regulators BA and NAA, and the best concentrations to produce the largest possible number of plants directly from the leaf were 2 mg L^{-1} of BA and 0.1 mg L^{-1} of NAA. l

Results showed clearly that the best treatment for callus induction from Anthurium leaves was MS medium containing a combination of 2.0 mg L^{-1} BA and 0.75 mg L^{-1} 2,4-D. The quantity as well as the quality of callus formation were highly increased at this concentration compared to other tested concentrations. Also, an indirect method through callus formed a larger number of plantlets than the direct method, which proved the advantage of propagation via callus. There is a synergistic effect between BA and 2,4-D on cell division and

differentiation, highlighted by these results; however, maximum efficiency necessitates growth regulator optimization in Anthurium tissue culture. l

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Novelty Statement

This work provides a new comparison evaluation of Anthurium differentiation through direct and indirect tissue culture pathways. It also contains information on the synergistic effects between BA and 2,4-D in callus induction as well as plantlet formation that could be used to optimise an inexpensive and fast propagation method for commercial growers of Anthurium.

Author's Contributions

Zainab, A.S. Mohammed and Omar H. Obaid: The authors carried out and supervised all aspects of the research activities from experimental design, plant tissue culture work, data collection and analysis, to statistical analysis and manuscript writing. Both authors equally contributed to conceptualization, interpretation of results, and preparation of the manuscript.

Generative AI and AI-assisted technology statement

The text has been worked and reworked exclusively by generative AI tools to make the language clearer and better formatted as a manuscript. All experimental results, analyses, interpretations, and scientific conclusions are original works of the authors. The AIs have not assisted or influenced in any way with the experimental design or data collection, or even results interpretation.

Conflict of Interest

There are no personal, financial, or institutional conflicts of interest related to this study between the authors or with any third party.

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