ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 06 | June - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

DEVELOPMENT OF AN EFFICIENT MICROPROPAGATION PROCEDURE FOR AGLAONEMA COSTATUM F. COSTATUM

Biotechnology

Dharmesh Dabasiya Post Graduate Student, Department of Life Sciences, Parul Institute of Applied Sciences, Parul University, At & PO – Limda, Waghodia, Vadodara 391760 Gujarat

Dr Saurabhkumar Mehta Assistant Professor, Department of Life Sciences, Parul Institute of Applied Sciences, Parul University, At & PO – Limda, Waghodia, Vadodara 391760 Gujarat

ABSTRACT

Aglaonema is a widely used ornamental plant, and one of the most sought-after kinds is the Snow White cultivar. To propagate this plant, an effective micropropagation method was created, which entails cultivating stem nodal segments in an aseptic culture using the Murashige and Skoog medium. The best conditions for axillary buds to grow were found when 4 mg/l of 6-benzyl adenine was added to the medium. After removing the single-stem nodal segments of the elongated shoots, different thidiazuron combinations were applied to them. With 1.5 mg/l thidiazuron, these treatments produced an average of 10.9 adventitious shoots per stem segment. The tiny shoot clusters underwent additional incubation with different ratios of thidiazuron and 6-benzyl adenine. The study discovered that 1.25 mg·l−1 thidiazuron and 4 mg·l−1 6-benzyl adenine treatments were more successful in promoting shoot elongation and proliferation. After Eight weeks, the longest shoots—4.7 cm—were grown on a medium containing 4 mg/l 6-benzyl adenine. Furthermore, the application of 1 mg/L indole-3-butyric acid was able to successfully run up to 90% of the elongated shoots in vitro. Ninety-five percent of the rooted shoots survived after being moved to the greenhouse. In summary, the Snow White cultivar of Aglaonema can be micropropagated with great efficiency, yielding an average of 10 adventitious shoots per stem segment. High rates of plantlet survival and rooting were attained during the research, which makes this technique beneficial for the large-scale propagation of this well-liked ornamental plant.

KEYWORDS

Micropropagation, Aglaonema, Plant Growth Regulators, Axillary bud, Adventious bud, 6-Benzyl Adenine, Thidiazuron, Indole-3-Butyric Acid, Shoot Multiplication, Rooting

1. INTRODUCTION

1.1 Aglaonema Costatum F. Costatum Cultivar 'Snow White'

The relationship between humans and ornamental plants dates back to the dawn of civilization. These vibrant companions were more than decorative; they signified wealth and were integral to cultural traditions throughout Latin America, Africa, and Asia. The Aglaonema, also known as the Chinese evergreen, is one such cherished plant. A member of the Araceae family, this genus includes many species indigenous to the moist forests of Southeast Asia [1], [2], [3]. The Aglaonema has earned its popularity as a houseplant with its beautiful leaves, low maintenance, and ability to thrive in low light and humidity [3]. With new varieties continually emerging, its global appeal persists [3]. Yet, commercial propagation of Aglaonema presents challenges due to its distinctive flowering pattern and the short life of its pollen, leading to reliance on cuttings and basal shoot division for reproduction. This method risks disease transmission if the parent plant carries pathogens in its vascular system [4]. Nonetheless, the allure of the Aglaonema is unmistakable. This monocotyledon features striking leaves on a spadix inflorescence. Its impressive array of colors, including shades of green, pink, red, and white, along with its versatility, secures the Aglaonema's place as a perennial favorite among ornamental plants [5]

1.2 Plant Tissue Culture and Micropropagation

Plant tissue culture is a valuable tool for plant scientists and horticulturists. It allows for the aseptic cultivation of plant cells, tissues, or organs on a defined medium under controlled conditions. This technique offers numerous applications, including studying plant growth and development, plant breeding, and micropropagation. Micropropagation is a specific type of plant tissue culture that focuses on the rapid clonal propagation of plants through the formation of adventitious shoots or buds. This method offers several advantages over traditional propagation techniques, including: Disease-free plants: Micropropagation takes place in a sterile environment, minimizing the risk of disease transmission. Rapid multiplication: Micropropagation allows for the production of a large number of plants from a single explant in a relatively short period. True-to-type plants: Micropropagation produces genetically identical clones of the parent plant, ensuring consistent characteristics in the offspring.

2. MATERIAL AND METHOD

2.1 Plant Material and Explant Preparation

Disease-free and actively growing shoots of Aglaonema costatum f. costatum cultivar 'Snow White' were used as the source of explants. Single-node segments were excised and surface-sterilized using established aseptic techniques.

2.1.1 Media Preparation and Culture Conditions

The Murashige and Skoog (MS) medium [6] served as the basal medium for all cultures. Various combinations of plant growth regulators (PGRs), including 6-benzyl adenine (BA), thidiazuron (TDZ), and indole-3-butyric acid (IBA), were added to the medium at different concentrations.

2.1.2 Aseptic Techniques

All instruments, containers, and media were sterilized in an autoclave at 121°C and 104 kPa. Culture vessels were specifically chosen clear plastic jars with deep-skirted lids to ensure proper containment. Each jar received 50 ml of the liquid media. A laminar flow cabinet was used to maintain aseptic conditions during critical transfers. Researchers donned sterile masks and gowns throughout the experiment.

2.1.3 Media Composition

Commercially available Murashige and Skoog (MS) medium (HIMEDIA, M&S Plant Salt Mix) [6] was chosen as the basal medium due to its well-established and effective combination of vitamins and minerals for plant tissue culture.

2.2 Sterilization Trial

Treatment 1: Sequential Fungicide and Antibacterial Soaks: The first treatment involved a series of sequential soaks targeting both fungal and bacterial contaminants. Following a preliminary 10-minute wash under running tap water to remove surface debris, explants were submerged in a fungicide solution containing mancozeb and metalaxyl (2 g/L) with the surfactant Tween-20 (10-12 drops) for 30 minutes. This step aimed to eliminate fungal growth. A second 30-minute fungicide soak followed, utilizing Azoxystrobin and tebuconazole (2 mL/L) for further fungal control. To eradicate bacterial contamination, explants were then immersed in Tagmycin (streptomycin sulfate and tetracycline hydrochloride, 1 g/L) for 30 minutes. Following each treatment step, the explants were thoroughly rinsed three times with sterile distilled water to remove any residual solutions.

Treatment 2: Modified Fungicide and Combined Antibiotic Treatment: The second treatment employed a modified approach with variations in fungicide and antibiotic selection. Similar to the first treatment, explants were initially washed under running tap water for 10 minutes. A single, modified fungicide treatment followed, utilizing Ridomil Gold fungicide (3 g/L) with Tween-20 surfactant (5-10 drops) for a 40-minute duration. To achieve broader bacterial control, a

combined antibiotic solution containing Tagmycin, Bavistin, Gentamicin sulfate, and Cefotaxime sodium (all at 1 g/L) with Tween-20 (5-10 drops) was employed for a 40-minute soak. Following this, the explants received a 40-minute copper oxychloride solution (1 g/L) rinse. A final bleach treatment with 20% sodium hydroxide, sodium hypochlorite, and amine oxide was applied for 10 minutes, followed by two rinses with sterile distilled water.

Final Steps: Alcohol Rinse, Mercury Chloride, and Drying

Both sterilization procedures converged for the final steps. The explants were sterilized with 70% isopropyl alcohol (IPA) for 30 seconds within a laminar flow cabinet to ensure aseptic transfer. This was followed by another rinse with sterile distilled water. As a final strong sterilization step, the explants were immersed in a mercury chloride ($HgCl_2$) solution (1 g/L) for ten minutes, followed by two final rinses with sterile distilled water. The explants were then carefully transferred to sterilized filter paper in a petri dish for drying.

2.3 Shoot Induction

2.3.1 Axillary Bud and Adventitious Shoot Elongation and Proliferation Induction

Each culture jar containing 50 ml of enriched MS medium was inoculated with a single stem node. The enriched medium included BAP and TDZ, PGRs known to influence shoot development. The experiment consisted of two culture cycles with seventeen replicates for each treatment combination. Cultures were maintained for four weeks under controlled conditions of 25°C ± 2°C temperature, a 12-hour light/dark cycle, and an average light intensity of 50 µmol·m-2·s-1 PAR. The number of leaves, shoots, and shoot height were monitored and recorded at two-week intervals. Specific details of PGR types and concentrations used for shoot induction are presented in Table 1.

Table 1: Composition Of Aglaonema Shoot Initiation Media Trials

Treatment	Base Constituents (per Liter)	Additional Constituents (per Liter)
AG1 Media	Full strength MS basal medium (34.4 g) + Gelrite (1.5 g) + Agar (1.75 g) (pH 5.8)	None
AG2 Media	Full strength MS basal medium (34.4 g) + Gelrite (2.2 g) + Agar (7.0 g) (pH 5.8)	BAP (1.0 mg) + TDZ (0.25 mg)
AG3 Media	Full strength MS basal medium (34.4 g) + Gelrite (1.5 g) + Agar (1.75 g) (pH 5.8)	BAP (2.0 mg) + TDZ (1.0 mg)
AG4 Media	Full strength MS basal medium (34.4 g) + Gelrite (1.5 g) + Agar (1.75 g) (pH 5.8)	BAP (4.0 mg) + TDZ (1.25 mg)

2.4. Root Induction of Adventitious Shoots for Plant Regeneration

Two basal media formulations derived from Murashige and Skoog (MS) medium were supplemented with various auxin combinations to assess their impact on root induction. Shoots, previously optimized for growth, were transferred to these media after a two-cycle, twelve-week multiplication phase on a shoot proliferation medium. Explants measuring 10-15 mm were selected for the rooting experiment. Each treatment group consisted of 17 replicates maintained for six weeks under controlled conditions. Data on root number, leaf number, shoot and root length was collected every two weeks. Details of the rooting media compositions are provided in Table 2.

Table 2. Root Induction Media Composition for In Vitro Culture

Treatment	Media	Plant Growth Regulators (PGRs)	Other Constituents
RT1	Full MS	IBA (1 mg/L) + AC (25 mg/L)	Sucrose (15 g/L), PPM (6 mL/L), pH 5.8
RT2	Full MS	IBA (1.5 mg/L) + AC (25 mg/L) + NAA (0.5 mg/L) + PBZ (0.04 mg/L)	Sucrose (15 g/L), pH 5.8
RT3	Full MS	IBA (2 mg/L) + NAA (1 mg/L) + AC (25 mg/L)	Sucrose (15 g/L), pH 5.8

2.5 Acclimatization:

Regenerated plantlets were acclimatized to ex vitro conditions in a greenhouse. A sterilized (autoclaved) potting mix with neutral pH (7.0) was used. Ex vitro rooting was induced by dipping shoot bases in IBA solution (prepared at a specific concentration) for 30 seconds. Each shoot was then planted in a pot containing a sterilized 1:1 peat moss-vermiculite mix. After eight weeks, root formation percentage, number of adventitious roots per shoot, and root length were evaluated. This method facilitates efficient acclimatization and plantlet production for various applications.

3. RESULT AND DISCUSSION

3.1 Sterilization

To prevent contamination by microorganisms like bacteria and fungi, sterilization is a crucial step throughout the tissue culture process. This typically involves using chemicals or physical methods to eliminate any microbes present on the explants (plant tissues used for culture initiation), culture vessels, and other materials. The provided text describes an experiment to identify the most effective sterilization procedure. The researchers tested two experimental chemicals on explants at various concentrations and exposure times. Presumably presented in Tables 3 and 4, the results indicate that treatment 2 was superior to treatment 1 in terms of achieving a sterile environment.

Tables 3 and 4: Initiation Material Contamination Assessment

These tables summarize the assessment of contamination in plant tissue culture initiation materials, specifically adventitious shoots and axillary buds. They capture the initial level of contamination before the first subculture.

Table 3: Effect Of Experimental Treatment 1 On Explant Sterilization

Initiation Material	Total No. of Clumps	Bacterial Contamination	Fungal Contamination	Total Contamination	No. of Clumps Remaining	Contamination Rate (%)
Adventitious Shoot	14	4	6	10	4	71.43
Axillary Bud	12	3	7	10	2	83.33

Table 4: Microbial Contamination Assessment Of Plant Initiation Cultures

Initiation Material	Total No. of Clumps	Bacterial Contamination	Fungal Contamination	No. Clumps Contaminated	No. Clumps Remaining	Percentage of Contamination Before 1st Subculture
Adventitious Shoot	14	1	1	2	12	14%
Axillary Bud	12	0	1	1	11	12%

Observations From the Tables:

- Axillary buds (83.33%) showed a higher contamination rate compared to adventitious shoots (71.43%) in Table 3, suggesting they are more susceptible to contamination during treatment 1.
- Both culture types displayed similar overall contamination levels (around 14%) in Table 4, with fungal contamination being slightly more prevalent.
- A significant portion of explants remained contamination-free (12 clumps each for both culture types) in Table 4.

3.2 Shoot Initiation In Aglaonema Costatum F. Costatum Shoots

In this section, the effects of various plant growth regulators (PGRs) on shoot initiation and elongation from stem nodal explants were investigated. After six weeks, tissues treated with Benzyl Adenine (BA) displayed significant development, with bud outgrowth observed in 90% of explants after two months. BA concentration positively influenced shoot growth rate and final length, with 4 mg/L BA resulting in the longest shoots (4.7 cm) compared to the PGR-free control (1.5 cm). Thidiazuron (TDZ) promoted adventitious shoot formation within four weeks, with shoots sometimes outgrowing the natural bud. Interestingly, multiple shoots of varying sizes could form on the same segment at different times. Notably, adventitious shoots emerged in all treatments, including the control. A specific combination of BA and TDZ, designated AG4 MEDIA (4 mg/L BA and 1.25 mg/L TDZ), proved statistically superior for shoot induction compared to the PGR-free medium and another BA-TDZ combination (AG MEDIA). AG4 MEDIA yielded the highest number of adventitious shoots per explant. Further analysis supported the differential effects of PGRs. BA promoted longer shoots (4.7 cm with 4 mg/L) compared to the control and displayed more variation in shoot length and number of leaves. Overall, PGRs significantly influenced shoot development. BA favoured axillary bud outgrowth and shoot elongation, while TDZ

induced adventitious shoot formation. The combination of BA and TDZ in AG4 MEDIA demonstrated the most promising potential for large-scale shoot multiplication.

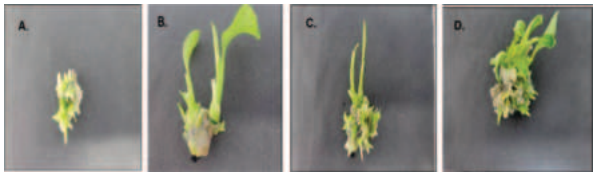

Figure 1: The impact of various PGR treatments on the proliferation and elongation of Aglaonema costatum f. costatum adventitious shoots after two months of culture.(A) control (B) 1/0.25 mg l−1 BAP/TDZ. (C) 2/1 mg·l−1 BAP/TDZ. (D) 4/1 mg·l−1.25 BAP/TDZ

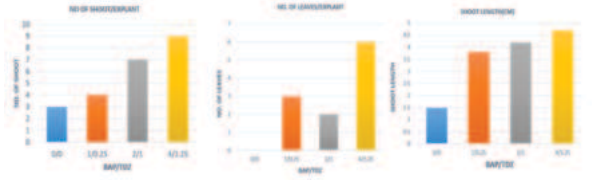

Figure 2: Effect of BAP and TDZ Concentration on Shoot Proliferation and Development in Aglaonema costatum f. costatum Micropropagation

3.3. Root Induction in Aglaonema Costatum F. Costatum Shoots

3.3.1 Effects of IBA and NAA on Adventitious Root Formation

This study investigated the influence of various Indole-3-butyric acid (IBA) and Naphthalene-acetic acid (NAA) concentrations on in vitro root formation (adventitious rooting) in Aglaonema costatum shoots. Shoots previously cultured on media containing 4 mg/L BA were used as the starting material. Three IBA/NAA combinations were tested: 1 mg/L IBA alone, 1.5 mg/L IBA with 0.5 mg/L NAA, and 2 mg/L IBA with 1 mg/L NAA. The highest number of roots per shoot (average of 6 roots) was observed in shoots treated with 1 mg/L IBA alone. Conversely, IBA/NAA combinations resulted in significantly fewer roots. Root length was only significantly greater in the 1 mg/L IBA treatment compared to other treatments. All treatments yielded a relatively high percentage of shoots forming roots (50% - 90%). Notably, 1 mg/L IBA alone resulted in the highest percentage (90%) of shoots forming roots. IBA alone was more effective for promoting root formation and growth compared to IBA/NAA combinations. The 1 mg/L IBA treatment yielded the most roots per shoot, longest roots, and the highest percentage of shoots forming roots.

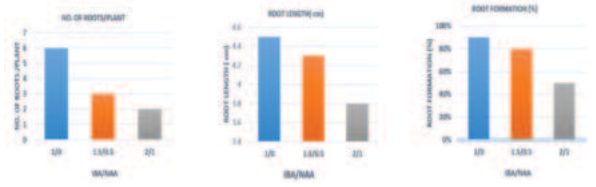

Figure 3: This graph depicts the effect of various Indole-3-Butyric Acid (IBA) and Naphthalene-Acetic Acid (NAA) concentrations on in vitro root formation in Aglaonema costatum f. costatum shoots.

IBA concentration has a positive effect on root number up to a certain point. The highest IBA concentration (2 mg/L) resulted in the fewest roots, suggesting an optimal IBA range exists. Root length appears less affected by IBA concentration across the tested values. IBA concentration seems to positively influence root formation percentage, with the highest concentration having the lowest percentage, possibly indicating an inhibitory effect of high IBA levels.

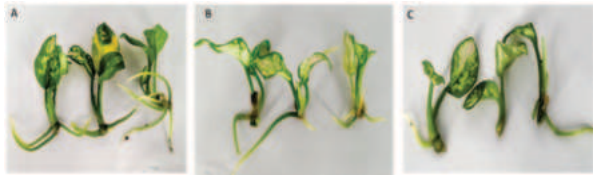

Figure 4: Influence of IBA and NAA on Root Number, Length, and Formation in Micropropagated Aglaonema costatum f. costatum Shoots. (A) 1/0 (B) 1.5/0.5 (C) 2/1 IBA/NAA concentration

3.4. Successful Acclimatization Of Micropropagated Aglaonema Costatum F. Costatum Shoots

This study achieved significant results in cultivating complete Aglaonema costatum f. costatum plantlets through in vitro micropropagation. Shoots were first elongated using a specific medium containing growth regulators, followed by root induction on a medium containing IBA (1 mg/L). This process yielded well-developed plantlets. The plantlets were then successfully acclimatized to the greenhouse environment, with a 100% success rate. Notably, 92.5% of rooted plantlets continued to thrive in the greenhouse, exhibiting healthy growth with 4-5 leaves within two months without fertilizer application .Interestingly, these acclimatized plants displayed a unique white variegation pattern on their leaves. A crucial finding was that the tissue-cultured plants were nearly indistinguishable from the mother plant in terms of vegetative features. This demonstrates the technique's effectiveness in replicating plants that maintain the desired characteristics of the original stock.

3.5 DISCUSSION

This research successfully established a reliable micropropagation protocol for the popular ornamental plant, Aglaonema costatum f. costatum. The traditional method of indirect organogenesis, which can lead to genetic variations, was bypassed by optimizing the type and concentration of plant growth regulators (PGRs) in the culture medium [7], [8]. The study emphasized the importance of sterilization to eliminate contaminants without harming the plant tissues. A balance between sterilization time and tissue viability was achieved through careful selection of disinfecting solutions and avoiding excessive durations (e.g., exceeding 40 minutes) [9], [10]. The culture medium, essential for plant growth in the controlled laboratory environment, was formulated using agar for solidification, essential nutrients (macro and micronutrients), vitamins, and sucrose as an energy source [11]. Murashige and Skoog (MS) medium proved to be a versatile and effective base for this purpose [11]. Plant growth regulators (PGRs) significantly influenced shoot initiation and development. Benzylaminopurine (BA) effectively promoted shoot growth from axillary buds, with higher concentrations leading to greater shoot numbers. Interestingly, no synergistic effect (combined positive effect) was observed between BA and thidiazuron (TDZ) on shoot elongation at the tested levels. However, a combination of BA and TDZ was successful in inducing adventitious shoot formation directly from the stem tissue. The most effective combination (4 mg/L BA and 1.25 mg/L TDZ) resulted in the highest number of shoots per segment compared to other treatments. Variations in shoot numbers were attributed to inherent differences in the stem segments, such as the number of meristematic cells present [12]Indole-3-butyric acid (IBA) proved to be a highly effective auxin for promoting adventitious root development due to its greater stability compared to other auxins [13], [14]. Root formation was achieved using 1 mg/L IBA in both in vitro and ex vitro conditions, with ex vitro cultures showing slightly better results. IBA remains the preferred auxin for Aglaonema shoots when prioritizing a high rate of root development.

4. CONCLUSION

This research successfully established a micropropagation method for rapidly multiplying Aglaonema 'snow-white' plants through adventitious shoot formation. The study identified an optimal sterilization process using a disinfectant solution for 40 minutes to prevent contamination without harming the plant tissue. Additionally, the most effective PGR combination (4 mg/L BAP and 1.25 mg/L TDZ) in the culture medium yielded an impressive average of 30 shoots per segment after 11 months. IBA, particularly at 1 mg/L, proved to be the superior auxin for promoting root development, generating more numerous, longer, and healthier roots compared to NAA. Notably, some root formation even occurred without any auxin application. Overall, this research provides a highly efficient and fast protocol for future Aglaonema micropropagation, paving the way for the development of novel Aglaonema cultivars with desirable traits through genetic transformation.

5. Acknowledgment

Dr Saurabhkumar Mehta, Assistant Professor, Department of Life Science for English review, guidance, and instructions. Mr. Narendra Mali, Sarjan Biotech Bhuj, for providing experimental plant material, comments, and support. Mr. Narendra Mali, MSc botanist, for literature collection and encouragement. Mr. Suraj Patil, fellow lab researcher and friend, for valuable advice. Mr. Harish Nagda, owner of Sarjan Biotech Bhuj- Kutch, for providing laboratory space. Dr Trilok AKhani, principal of Applied Science, Parul University for infrastructure and resources. Dr Gireesh Babu K, head of the Department, and all faculty members for their support. Friends for

research discussions, fun, and support. Family, especially parents, for their encouragement, inspiration, love, concern, and support.

REFERENCES

[1] A. hay, "A new species of Aglaonema Schott (Araceae)," vol. 50, Terengganu, Malaysia: the gardens bullentin, Singapore, 1998, pp. 1–4.
[2] Huxley A, the New Royal Horticultural Society Dictionary of Gardening, vol. 30, no. 06. London: Macmillan Press Ltd, 1994.
[3] J. Chen, R. J. Henny, and D. B. McConnell, "Development of New Foliage Plant Cultivars," no. August, Portland: Timber press, 2002.
[4] Chase AR, "Foliage Plant Diseases: Diagnosis and Control," in Foliage plant diseases: diagnosis and control, no. 2, Saint Paul, Minnesota: American Phytopathological Society, 1997, pp. 8–11. Doi: 10.1023/a: 1010205005039.
[5] Totik Sri Marian, Any Fitriani, Teixeira da Silva, Adhityo Wicaksono, and Tet Fatt Chia, "Micropropagation of Aglaonema using Axillary Shoot Explants," International Journal of Basic & Applied Sciences, vol. 11, pp. 46–53, 2011.
[6] M. K. Razdan, Introduction to plant tissue culture, vol. NA, no. NA. 1993.
[7] X. Shen, J. Chen, and M. E. Kane, "Indirect shoot organogenesis from leaves of Dieffenbachia cv. Camouflage," Plant Cell Tissue Organ Cult, vol. 89, pp. 83–90, 2007, doi: 10.1007/s11240-007-9214-7.
[8] R. J. Henny and J. Chen, "Tropical Foliage Cultivar Development: New Ornamental Introductions Drive Plant Markets," EDIS, vol. 2008, no. 2, 2008, doi: 10.32473/edis-ep357-2008.
[9] K. C. Torres, Tissue Culture Techniques for Horticultural Crops. New York. Van Nostrand Reinhold, 1988. Doi: 10.1007/978-1-4615-9756-8.
[10] H. C. Sweet and W. E. Bolton, "The Surface Decontamination of Seeds to Produce Axenic Seedlings," Am J Bot, vol. 66, pp. 692–698, 1979, doi: 10.2307/2442414.
[11] Murashige T & Skoog F, "A revised medium for rapid growth and bioassays with tobacco tissue cultures," Physiol. Plant, vol. 15, pp. 473–497, 1962.
[12] M. K. Kim, H. E. Sommer, B. C. Bongarten, and S. A. Merkle, "High-frequency induction of adventitious shoots from hypocotyl segments of Liquidambar styraciflua L. by thidiazuron," Plant Cell Rep, vol. 16, no. 8, 1997, doi: 10.1007/bf01142319.
[13] JF Hutchinson, "Tissue culture of propagation of fruit tree." Singapore: Proceedings of the international symposium on tissue culture of economically important plants., 1981, pp. 113–120.
[14] M. T. Jahan, M. R. Islam, R. Khan, A. N. K. Mamun, G. Ahmed, and L. Hakim, "In vitro clonal propagation of Anthurium (Anthurium andreanum L.) Using callus culture," Plant Tissue Culture and Biotechnology, vol. 19, pp. 61–69, 2009, doi: 10.3329/ptcb.v19i1.4961.