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MICROCLONAL PROPAGATION TECHNOLOGY OPTIMIZATION OF *AGLAONEMA* CV. SILVER BAY

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SUMMARY

The *Aglaonema* cultivar Silver Bay is distinct with its highest cost compared with other cultivars. In previous years, the necessity of research aimed at optimizing plant propagation processes has been increasing. The consequent study sought to conduct experiments on the optimization of the different stages involved in microclonal propagation of this important plant. Initially, clarifying the sterilization conditions of plant materials succeeded. Sterilization of materials in Domestos for 20 minutes, in 70% ethanol for two minutes, and in 0.1% $AgNO_3$ solution for three minutes allowed the increase in viability to 87.6%. Moreover, to enhance the efficiency of multiplication and rooting stages through microclonal propagation, standards for enriching the Murashige and Skoog nutrient medium with benzylaminopurine (BAP) and metatopolin were options. This tested combinations of substrates that increase the viability of regenerants at the rooting stage. Under selected optimal conditions, the number of shoots formed by explants was 2.6, reducing the emergence of shoots to 8.2 days, having root length at the fifth week of rooting at 6.7 cm, and the viability of seedlings during acclimatization reaching 89.4%. The obtained results could serve to increase the diversity of methods used in the propagation of indoor plants.

Keywords: *Aglaonema,* benzylaminopurine (BAP), cultivar Silver Bay, indole-3-butyric acid (IBA), in vitro, microclonal propagation, Murashige and Skoog, sterilization

Key findings: Research into optimizing the stages of microclonal propagation of *Aglaonema* has provided scientific solutions for increasing the efficiency of seedling production. The results obtained will serve to improve methods for mass propagation of ornamental plants.

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INTRODUCTION

In the present era, the propagation of household ornamental plants using modern technologies is providing better results worldwide. *Aglaonema* is a genus of evergreen plants belonging to the family Araceae, widely used as ornamental and interior decoration plants. This genus comprises more than 21 species and has reached cultivation in Asian countries for centuries as a symbol of good luck (Utekar *et al*., 2023).

Aglaonema species mostly prefer relatively low and indirect light levels, and therefore, they can also grow well indoors under artificial lighting. Growing these ornamental plants commonly occurs in areas, such as waiting rooms, lobbies, and office buildings, where these plants can easily flourish in existing environments with 10%–25% light. Such types of plants include valuable variegated leaf cultivars that are distinctive with their beautiful leaf shape, various colors, sizes, patterns, drought tolerance, and tolerance to relatively low humidity (Yeh *et al*., 2007).

In general, the in vitro microclonal propagation process involves four primary steps. First, the explants received surface sterilization before being placed in an initial nutrient medium. Second is the propagation stage, where explants undergo propagation by culturing them in new media several times until reaching the required size. Third, the root formation stage requires the transfer of explants to a special medium to perform root induction. Finally, the fourth and last is the adaptation stage, where growing the seedlings in vitro to adapt to an ex vitro environment (Hussain *et al*., 2012). However, the successful development of plant seedlings in vitro depends on various factors, including the composition of the medium, environmental conditions, and especially the plant species.

The types of phytohormones that control plant growth and development and their concentrations mainly incur determination according to the plant species, plant organs, and tissues used, as well as the entire purpose of cultivation (Mongkolsawat *et al*., 2023). Additionally, the type of explant cultivation system also directly and considerably affects the efficiency of in vitro conditions. According to past research, liquid culture media proved to be more cost-effective in the in vitro propagation process than in solid and semi-solid gel culture media (Paek *et al*., 2005).

Aglaonema are the perennial plant species that produce roots from the joints of their upright and curved stems. Their leaves have unique color combinations, such as green-orange, green-red, green-yellow, green-white, green-pink, and silver. The characteristic inflorescence of these species is a raceme (Mariani *et al*., 2015). Sexual reproduction is a complex process, as mostly the flowers do not open simultaneously. The lifespan of pollen cells is short, and overall, the generations entail segregation genetically. Therefore, most *Aglaonema* species mainly succeeded in vegetative propagation by cuttings taken from the joints of the stem and by dividing the lower parts of the shoot (Barakat and Gaber, 2018).

Generally, the microclonal propagation allows the improvement of new cultivars in a short time before reaching the level of commercial production. Furthermore, the lowest rate of plant reproduction and slow and steady growth are also critical problems. Nevertheless, the in vitro plant propagation provides physiologically stable growth and development and allows for rapid propagation (Utekar *et al*., 2023).

The microclonal propagation carried out in the *Aglaonema* cultivar Valentin has significantly reduced the time for plant propagation. That study aimed to determine the optimal amounts of growth regulators for healthy and proper growth of seedlings grown by manipulating *Aglaonema* cells, tissues, and organs in a nutrient agar medium enriched with special growth regulators (Sawardeka and Sandip, 2024). Microclonal propagation of apical buds of the species *Aglaonema simplex* in the MS medium supplemented with doses of naphthalene acetic acid (NAA) (0.5 mg/l) and BAP (0.5 mg/l) was considerably optimal (Laohavisuti and Mitrnoi, 2005). In another study, the explants developed well by growing in the MS medium augmented with BA (30 µM). However, better results were noteworthy

when deliberately combining the MS medium with 20 µM thidiazuron (TDZ) (Chen and Yeh, 2007).

In vitro, the plants' lateral shoot growth showed better results in medium supplemented with BA (10 mg/l) (Fang *et al*., 2013; Rayimova *et al*., 2024). In past studies, the most effective and optimal nutrient media emerged by having the following composition: MS + 2.0 mg/l 6-BA + 0.2 mg/l IBA as the most optimal medium for inducing lateral shoots; MS + 0.5 mg/l TDZ + 2.0 mg/l 2,4-D as the optimal medium for callus formation; and 1/2 MS + 0.5 mg/l TDZ appearing as the most effective nutrient media for inducing shoot set (Zhou *et al*., 2018; Alikulov *et al*., 2022a). Based on the above different analyses, the presented study aimed to improve the in vitro microclonal propagation process of the *Aglaonema* cultivar Silver Bay. This included obtaining explants, determining the optimal sterilization method, and selecting conditions for attaining massive healthy regenerant seedlings using different combinations of growth regulators.

MATERIALS AND METHODS

Research objects

The research commenced at the Molecular Biotechnology Laboratory of Samarkand State University named after Sharof Rashidov. Dan Henry Nicholson's fundamental study, "A Reconsideration of the Genus *Aglaonema* of the Family Araceae," published in the Smithsonian Contributions to Botany in 1969, centered on a comprehensive taxonomic analysis of the genus *Aglaonema*. The said study covered areas in the regions from Northeastern India to New Guinea. The conduct of the research was according to more than 1500 herbarium specimens stored in 33 herbaria, as well as field studies conducted in Southeast Asia and in more than 100 personal collections collected by the said researcher. The study also included a detailed analysis of previous literature on the genus, in particular, cytology, embryology, and morphology. As a result, the sections *Aglaonema* and *Chamaecaulon* received distinction for the first time on a scientific basis. Although 76 binomial names have previously succeeded in their publication for the genus *Aglaonema*, recognition of only 21 names emerged as valid and independent species in this study (Dan, 1969) (Figure 1).

Preparation and isolation of primary samples

Disease-free, healthy, and one-year-old *Aglaonema* cv. Silver Bay plants grown in a greenhouse sustained selection as mother plants. Mother plants entailed maintenance in the greenhouse protected from 50% sunlight, following all recommended agrotechnical practices. Irrigation stopped one week before the apical meristems of the stems used as explants reached isolation. In reducing

Figure 1. Research object: Cultivar Silver Bay of *Aglaonema*.

Table 1. Sterilization agents and time.

Sterilization procedure	Sterilization options (minutes)			
	I	II	III	IV
Wash in distilled water	30	30	30	30
Wash in Domestos (NaOCl) solution	10	15	20	25
Ethyl alcohol (C2H5OH), (70%)	1	1.5	2	2.5
Silver nitrate (AgNO3), (0.1%)	1	2	3	4

contamination by pathogens, the treatment of mother plants by spraying a solution of Carbendazim fungicide (3.0 g/l) ensued. According to the recommended methodology, the collection of explants occurred early in the morning. After washing in tap water, removing the leaves preceded the separation of leaf sheaths and the opening of dormant buds at the joints. The cutting of explants into 2.5–3.0 cm long cuttings followed before submitting for the sterilization process (Ulugbek *et al*., 2025).

Sterilization of material

The use of four sterilization options served to sterilize the starting material—apical meristems obtained from the *Aglaonema* cv. Silver Bay (Table 1). Sterilization efficiency assessment continued by determining the viability of explants of the input materials (Khasanov *et al*., 2023).

Cultivation conditions

The glass jars with the plant material remained in a special growth chamber with a humidity of 60%, 22 °C ± 1 °C/16 h light, and 15 °C ± 2 °C/8 h dark. The light intensity was 2600 lux. White fluorescent lamps served as the light source (Salama, 2021).

Multiplication stage

Utilizing the MS medium containing benzylaminopurine (BAP) and metatopolin (MT) at different concentrations helped multiplication (Murashige and Skoog, 1962). Each germinated explant underwent planting in a separate glass jar. The transfer of microclones to a new medium occurred every 21 days. The process efficiency evaluation continued by assessing the number of microshoots formed and the duration of their formation (Barakat and Gaber, 2018; Ergasheva *et al*., 2024).

Rooting stage

Healthy microshoots (3–4 cm long) with good quality traits were options for rooting. The hormone indole-3-butyric acid (IBA) used in different concentrations sought to evaluate its effect on the root formation in microclones and determine the optimal amount of the hormone. The process efficiency assessment continued by determining the average number of roots and average root length (Mariani *et al*., 2015; Akramov *et al*., 2025).

Acclimatization stage

The determination of the efficiency of adapting *Aglaonema* cv. Silver Bay seedlings to the substrate selected three different substrate options using available resources: a) peat and vermiculite in a ratio of 1:3; b) peat and vermiculite in a 1:1 ratio; and c) peat and vermiculite in a ratio of 3:1. Explants planted in 104 deep cassettes filled with substrate and shoot length and number of surviving explants entailed calculations (Utekar *et al*., 2023).

Statistical analysis

All the collected data based on various parameters attained analysis using OriginPro (2021) and Excel programs based on mean values and SE indicators (Alikulov *et al*., 2022b, 2023).

RESULTS

Sterilization

The sterilization process comprised four consecutive stages, with the processing time gradually increasing in each stage, depending on the different options. In the first stage, using the same conditions for all options took place, with the explants washed in distilled water for 30 minutes. This process served to reduce mechanical contamination and microorganisms on the surface. In the second stage, the explants received washing with the Domestos (NaOCl) solution for different periods of time. In option I, the sterilization continued for 10 minutes; in option II, it took 15 minutes; for option III, it was 20 minutes; and in option IV, it was 25 minutes. That stage sought to eliminate the majority of microorganisms, and the degree of sterilization enhanced with increasing time. In the third stage, the use of 70% ethyl alcohol (C_2H_5OH) proceeded, with the treatment time increased from 1 to 2.5 minutes. Alcohol treatment served to quickly neutralize surface bacterial and fungal spores. In the fourth and final stage, the explant sterilization in a 0.1% silver nitrate ($AgNO_3$) solution ran for 1–4 minutes. Silver nitrate has a considerable and robust antibacterial and antifungal effect, further increasing the efficiency of sterilization. During this study, the third sterilization option emerged as the most optimal option for sterilizing *Aglaonema* cv. Silver Bay apical meristems. Accordingly, the explants' survival rate was 87.6%.

Obtaining mother material

Explaining this phase was the presence of a single apical meristem bud at the stem tips, while at the joints appeared 3–4 dormant lateral buds (Figure 2). The said figure details the specific stages of explant development in the MS culture medium during microclonal propagation in vitro. At the initial stage of the experiment, the explants isolated from the apical meristem adapted to the culture medium and maintained their viability, showing initial signs of regeneration. However, in the apical meristems, the shoot formation was relatively slow. In later stages of cultivation, the growth and development of apical shoots and the activation of several dormant lateral shoots at the different joints were evident. This phenomenon's explanation refers to the increased activation of shoots with the influence of the composition of the MS nutrient medium and phytohormones.

The enhancement in the number of shoots and elongation of the stems further allowed the formation of explant balls, demonstrating the highest efficiency of the microclonal propagation process. The formed explant sets were recognizably the promising material for subsequent stages of subculturing and root formation.

Multiplication

In microclonal propagation of *Aglaonema* cv. Silver Bay, the results revealed the level of explant formation in all nutrient media with the addition of growth regulators was significantly

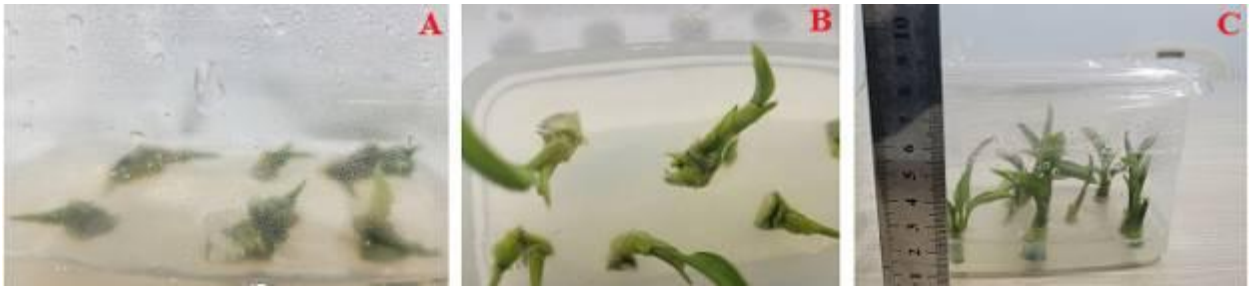

Figure 2. The efficiency of the formation of mother materials of the *Aglaonema* cultivar Silver Bay on MS medium: a) apical meristem buds, b) lateral shoot formation, and c) explant bulb formation.

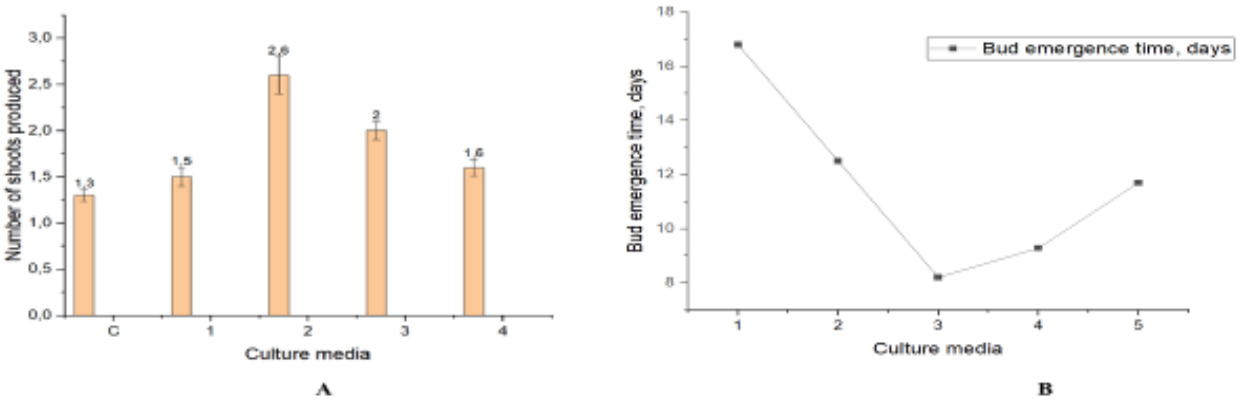

Figure 3. Performance indicators of the multiplication stage of microclonal propagation of *Aglaonema* Silver Bay cultivar (n=5): A) Number of shoots produced, and B) Bud emergence time, days (1 = MS (control); 2 = MS+BAP 0.4 mg/l + MT 0.2 mg/l; 3 = MS+BAP 0.8 mg/l + MT 0.5 mg/l; 4 = MS+BAP 1.2 mg/l +MT 0.8 mg/l; and 5 = MS+BAP 1.6 mg/l +MT 1.1 mg/l),

higher than in the MS medium without growth regulators (control). Among the treatment variants with growth regulators, the variant with the addition of 0.8 mg/l BAP + 0.5 mg/l metatopolin to the MS medium showed promising results. The number of shoots formed by explants was the largest (2.6), making that variant distinguishable from other options by the earliest emergence of shoots (8.2 days) (Figure 3).

In explants grown in MS control nutrient media without growth regulators, the shoot formation was relatively low (1.02), which also took a longer time for the shoot to form (16.8 days). In variant 1, with the concentration of 0.4 mg/l BAP + 0.2 mg/l metatopolin, the bud formation time was relatively short (12.5 days), with nonsignificant variation observed in the number of buds (1.07). For the second variant, with increased concentration of phytohormones (MS + BAP 0.8 mg/l + metatopolin 0.5 mg/l), better results were notable—the number of buds was 2.60, on average, with the bud formation period reduced to 8.20 days.

At the higher concentrations of BAP and metatopolin, a relative decrease resulted in the number of buds, recording a further extension in the bud formation period. In particular, the third and fourth variants (1.94 and 1.53, respectively), having the highest content of phytohormones, have a certain inhibitory effect on the growth and development processes. According to study results, the combination of BAP (0.8 mg/l) and metatopolin (0.5 mg/l) in the MS medium succeeded in their selection as an optimal option for rapid and abundant bud formation in the *Aglaonema* cv. Silver Bay.

Rooting

The results enunciated that the increase in the rooting rate of explants depended on the concentration of auxins in the culture medium, which provided root formation. However, it was also noticeable that higher concentrations of auxins can reduce the rooting rate (Figure 4). In the culture medium with different concentrations of IBK (indolyl-3-butyric acid), the study of the root formation process proceeded. In the control variant (0.0 mg/l IBK), no root formation appeared during the first three weeks, and only in the 4–5 weeks did a very few roots emerge, with an average index of 0.12. The results revealed that root induction was significantly slow in the culture medium without IBK. By using the IBK at the concentrations of 0.5 and 1.0 mg/l, the root formation process began at 3–4 weeks. The rooting intensity was relatively low, and the average index was 0.60 and 1.02, respectively.

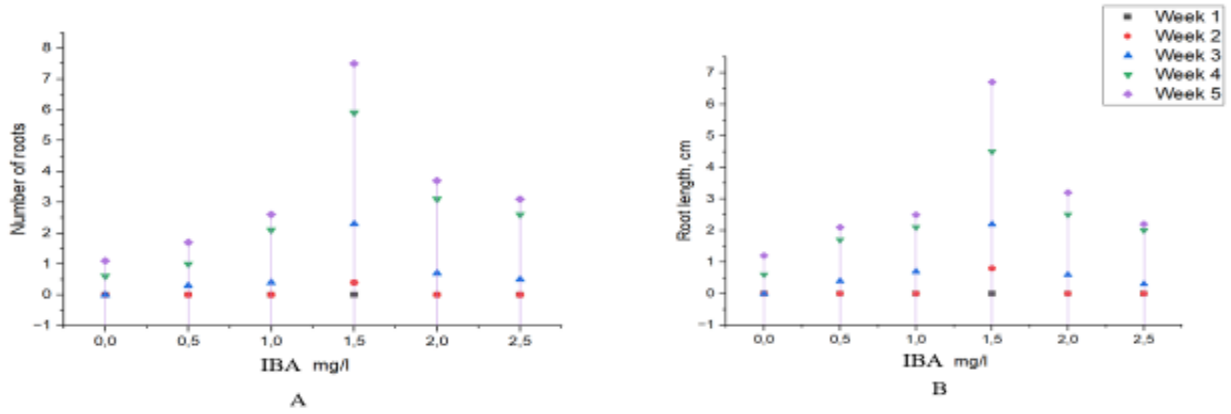

Figure 4. Rooting stage efficiency indicators of microclonal propagation of *Aglaonema* cultivar Silver Bay (n=5): A) number of roots and B) root length (cm).

The above concentrations stimulated the rooting process; however, they did not provide the highest and most desirable efficiency.

According to the results, the highest rooting indices recorded occurred at the concentration of IBK (1.5 mg/l). In this variant, the root formation began at 2–3 weeks, and rooting sharply increased at 4–5 weeks, reaching 5.9 and 7.5, with an average rooting index of 3.22. The results exhibited that the concentration of IBK (1.5 mg/l) activated cell division in explants, increased the root induction, and provided an optimal balance for their growth and development. By increasing the concentration of IBK (2.0 and 2.5 mg/l) in the plant diet, a significant decrease was evident in rooting traits. In particular, the average indicators were 1.50 and 1.24, respectively. At these highest concentrations, the slowing down of root development was a result of the inhibitory effect of IBK (Figure 4A).

The results expressed that the effect of IBK concentration on root length significantly varied. In particular, in the variant with IBK (1.5 mg/l), the early root formation manifested, recording the highest root length. In this variant, the root length at week 5 was 6.7 cm, with an average value of 2.84 cm. At low concentrations of IBK (0.0 and 1.0 mg/l), the root development received significant inhibition, while at the highest IBK concentrations (2.0–2.5 mg/l), an inhibitory effect appeared on root growth (Figure 4B). This may refer to the physiological stress induced by IBK with higher doses. The final stage of in vitro plant propagation was the adaptation of regenerants to non-sterile conditions. However, several difficulties were noteworthy at this stage, namely, the morpho-anatomical characteristics of seedlings, heterotrophic nutrition, variations in humidity, poor development of the mesophyll of the root system, low photosynthetic activity, and the incomplete transpiration.

The presented study took place in accordance with generally accepted methods under sterile conditions, from the stages of planting *Aglaonema* cv. Silver Bay explants in vitro in a nutrient medium to the preparation of rooted seedlings. Therefore, it was crucial to ensure that the substrate prepared for planting rooted seedlings in greenhouse conditions was also sterile. For full adaptation of explants and better development of the aboveground part and root system, it was particularly important that the substrate be well permeable to air, retain the necessary moisture, and not damage the plant roots during transplantation.

Acclimatization

In the first option, the seedling length was low (3.2 cm), and accordingly, its viability was also low (Table 2). In the substrate, the highest proportion of vermiculite ensures a high amount of air in the environment; however, a decrease in the peat amount leads to

Table 2. Performance indicators of the acclimatization stage of regenerative seedlings of *Aglaonema* cultivar Silver Bay*.

Substrate	Seedling length (cm), n = 20	Explant viability (%), n = 10
Peat (P): Vermiculite (V) – 1:3	3.2±0.1	23.6
P:V – 1:1	4.3±0.3	53.2
P:V – 3:1	7.4±0.4	89.4
P:V – 1:2	3.8±0.3	60.5

*Note. $P \leq 0.05$

insufficient moisture and nutrients. This may be because of the slow growth and inability of seedlings to adapt. Option 3 of the peat and vermiculite substrate appeared considerably optimal as it revealed the highest effect on seedling growth and explant viability. The average length of the seedlings was 7.4 cm, which was significantly higher than all other options. The results indicated that this substrate developed favorable conditions for the vegetative growth and development of the seedlings, along with increased viability of seedlings (89.4%).

DISCUSSION

For in vitro propagation of the *Aglaonema* type Dud Anjamani, the plant apical meristems received effective surface sterilization with a mixture of 0.5 g/L mercuric chloride ($HgCl_2$) and 2.0% sodium hypochlorite (NaOCl) (Salama, 2021). By sterilizing *Jasminum nudiflorum* explants and keeping them in a 1.0% $NaOCl_2$ solution for 10 minutes, followed by 70% ethanol for 10 seconds, provided the highest culture survival rate (63.88%) and optimal aseptic conditions (63.88%). The next most effective method was the treatment with a 0.1% $HgCl_2$ solution for 10 minutes, followed by 70% ethanol for 10 seconds, which revealed the culture preservation of 61.11%, and the aseptic condition was 69.44% (Showkat *et al*., 2022).

With optimal sterilization options during microclonal propagation of *Aglaonema* cv. Silver Bay, the highest rate of meristem culture formation was successful by performing the same treatment sequence on both types of explants in the following order. The explants received initial pretreatment with a mixture of Carbendazim (0.2%) + Mancozeb (0.2%) + Plantomycin (200 ppm—parts per million) + Tween-20 for two hours. Afterward, surface sterilization continued by successively treating with 4% NaOCl solution for 30 minutes, 0.1% $HgCl_2$ solution for 15 minutes, and 80% ethanol solution for one minute. In the final step, the shoots remained in Cefotaxime (200 ppm) solution for 30 minutes before sterilization by washing with distilled water in the final step. The survival rate of meristem tissues was the highest, allowing for the production of stable explants (Karthik *et al*., 2023).

Additionally, the tight covering of the apical meristem by young leaves located near the stem tip may have prevented rapid bud growth. Several buds also evidently sprouted from the lateral axils within a short time. Commercial propagation of *Aglaonema* from axillary buds has also attained confirmation in numerous studies (Barakat and Gaber, 2018; Kaviani *et al*., 2019; El-Gedawey and Hussein, 2022).

The cytokinins and auxins' remarkable role in microclonal propagation was well-studied, and the combination of cytokinins and auxins exerts a synergistic effect on the morphogenesis process, ensuring maximum results. Barakat and Gaber (2018) also reported the combination of cytokinins with auxins boost the number of shoots per explant compared with using BAP alone (Barakat and Gaber, 2018).

Philodendron Pink Princess underwent in vitro propagation in solid MS-medium supplemented with various concentrations of 6-benzylaminopurine (BAP) and naphthalene acetic acid (NAA), revealed the highest number of shoots and leaves surfaced at the concentration of BAP (1.0 mg/l). Moreover, the shoots gained a significant increase in plants grown in the liquid MS medium supplemented

with BAP (1.0 mg/l), with an average of 11.2 shoots and 4.7 leaves per explant. During the rooting stage, the most effective concentration of indole-3-butyric acid (IBA) in solid MS medium supplemented with auxins (3 mg/l) resulted in an average of 3.2 roots and 1.9 cm long per explant (Klanrit *et al*., 2023).

For in vitro microclonal propagation of *Aglaonema commutatum*, the MS medium supplemented with 1.0 mg/l BA + 2.0 mg/l NAA resulted in the development of primary shoots in 80% of explants after the fourth week. During shoot propagation stage, most effective MS medium supplemented with 4.0 mg/l BA + 1.0 mg/l NAA resulted in three shoots per dish. At the root stage, an average of five roots developed per explant in the MS medium supplemented with 0.5 mg/l IBA + 0.25 mg/l NAA (Swaranjali and Abhishek, 2023). During microclonal propagation of *Aglaonema* species, the cytokinin BAP concentration (0.25 to 5.0 mg/l) proved to be more effective (Utekar *et al*., 2023).

The MS medium at full concentration and with the influence of phytohormones, such as BA, NAA, and IBA, plays an important role in the processes of organogenesis and rhizogenesis (Utekar *et al*., 2023). *Aglaonema* species propagated in vitro maintained 100% survival in a 3:1 mixture of peat and sand for a greenhouse adaptation (Salama, 2021). One such promising indoor flower, Philodendron Pink Princess, showed the maximum survival rate with a combination of three substrates, viz., peat moss, vermiculite, and perlite (Klanrit *et al*. 2023).

CONCLUSIONS

In microclonal propagation of *Aglaonema* cv. Silver Bay, sterilization of the starting material in Domestos for 20 minutes, in 70% ethanol for two minutes, and in 0.1% $AgNO_3$ solution for three minutes, led to the highest level of viability (87.6%). In this plant, the enrichment of the MS nutrient medium with BAP (0.8 mg/l) and metatopolin (0.5 mg/l) enhanced the efficiency of the multiplication stage, while enrichment with IBA (1.5 mg/l) increases the efficiency of the rooting process. During the acclimatization of regenerant plant seedlings under greenhouse conditions, the use of peat and vermiculite substrates in a 3:1 ratio increases viability.

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