2023 International Research Conference of Sri Lanka Technology Campus
Colombo, Sri Lanka. 14th-15th December 2023

A Review in Micropropagation of Ornamental Aquatic Plants

R.M.P.I. Rajapaksha,
Department of Agriculture
Faculty of Technology
Sri Lanka Technological Campus
Padukka, Sri Lanka
isharar@sltc.ac.lk

R.G.J. Perera
Department of Agriculture
Faculty of Technology
Sri Lanka Technological Campus
Padukka, Sri Lanka

D.D.N. Vibodhani
Department of Agriculture
Faculty of Technology
Sri Lanka Technological Campus
Padukka, Sri Lanka
nisansalav@sltc.ac.lk

H.D.T. Abeyrathna
Department of Agriculture
Faculty of Technology
Sri Lanka Technological Campus
Padukka, Sri Lanka
diliniab@sltc.ac.lk

N.D.G.K.N. Jayawardhane
Department of Agriculture
Faculty of Technology
Sri Lanka Technological Campus
Padukka, Sri Lanka
kanchanaj@sltc.ac.lk

R.M.S.M.B. Rathnayaka
Department of Agriculture
Faculty of Technology
Sri Lanka Technological Campus
Padukka, Sri Lanka
sangeethr@sltc.ac.lk

***Abstract*—Ornamental aquatic plant production is currently gaining much commercial attention, not just for its aesthetic value, but also for its other benefits. These benefits include preserving water quality in aquariums, improving water clarity and quality, reducing rates of shoreline erosion and sediment resuspension, and aiding in halting the spread of invasive alien plant species. These plants also provide important habitats for fish and wildlife in their natural environments. However, conventional plant propagation methods such as seed, stem fragments, root crowns, and rhizomes have their issues. These issues may hinder the effectiveness of commercial ornamental aquatic plant production. Micropropagation techniques can overcome these issues, and researchers have been investigating preferable protocols for these plants for the past few decades. Nowadays, new technologies are being evaluated to improve these protocols. This review aims to identify effective protocols suggested for some popular aquatic plants.**

Keywords—Ornamental aquatic plants, explant preparation, shoot and root induction, acclimatization

I. INTRODUCTION

Clonal propagation is widely used in crop production to preserve the desirable characteristics of specific genotypes or varieties in the progeny. Due to the process's cutting back, *in vitro* clonal plant propagation is also known as micropropagation [1]. By utilizing plant tissue culture techniques, micropropagation is a means of quickly growing stock plant material to generate a large number of offspring plants [2]. Plant tissue culture is a method for regenerating and propagating complete plants by cultivating isolated plant cells, tissues, and organs *in vitro* under axenic conditions. All plant cultures, including callus, cell, protoplast, anther, meristem, embryo, and organ cultures, are collectively referred to as "tissue cultures" in common parlance [3]. Plants are totipotent, meaning they can regenerate entire plants from individual cells or groups of cells within a tissue or organ, expressing the entire plant genome, in contrast to most animal cells [4]. That is the major reason plants can be regenerated under *in vitro* conditions. The success of plant regeneration via micropropagation depends on several factors such as explant, culture media, sterilizing agents and their application rates, and types of plant growth regulators used [2]. Currently, the most practical and economically efficient plant biotechnology is micropropagation, which quickly produces many clonal plants of many different plant species [4].

Compared to the traditional vegetative and reproductive methods available for plant propagation, micropropagation techniques possess more beneficial characteristics. Since cultures are started with extremely small pieces, maintaining or significantly increasing the number of plants only requires a small quantity of room. The disease incidences can be minimized because aseptic environmental conditions are provided throughout the period. Also, it is feasible to modify the variables that affect vegetative regeneration more flexibly, including temperature, light, and the concentrations of growth regulators and nutrients. As a result, a far higher propagation rate than with macro propagation is possible, resulting in the production of many more plants in a given amount of time. Another crucial factor is the potential to quickly and extensively introduce new technologies or newly bred plants and selections to the market. Other than that, less labor requirement, less energy and space requirement, output is more resilient to seasonal variations and can be carried out year-round, and clones of certain plant species that are otherwise slow, hard, or impossible to replicate vegetatively may be created [4], [5]. Currently, several plant species and varieties are being propagated through these techniques to attain the above-mentioned benefits. Among them, flowers and ornamental species (Orchids, Anthuriums), fruit crops (Banana,

Pineapple), plantation crops (Sugarcane), and vegetable crops play a major role [4].

The adaptations of aquatic plants allow them to survive in freshwater or saltwater settings. They can be divided into three groups: floating, bunch, and rooted plants. They are also known as hydrophytes or macrophytes. In the ornamental fishing sector, aquatic plants are important because they provide a pleasing appearance to the observation units and a variety of benefits for the occupants [6]. In addition to its aesthetic value, ornamental aquatic plants are used to preserve water quality in aquariums. These are chosen based on plant color, size, and shape [7]. In their natural habitats, aquatic plants can enhance water clarity and quality, lower rates of shoreline erosion and sediment resuspension, and aid in halting the spread of invasive alien plant species in addition to providing important habitat for fish and wildlife [8].

There are primarily two kinds of aquatic plant propagation techniques identified as asexual and sexual propagation methods [6]. Among them, seeds belong to sexual propagation and under the asexual propagation, propagules such as stem pieces, root crowns, or winter buds or tubers, which are dormant perennating organs can be identified [6], [8]. Several aquatic plant species are propagated through seeds or by division of rhizomes which is not a productive method and also it takes a longer time [7]. These facts lead to huge issues in commercial plant production. Due to their weakness, these propagules need almost perfect circumstances in order to be successfully established in artificial reservoirs. The majority of artificial reservoirs are doomed to failure in their tough surroundings. Another thing is that the natural production of some of the propagules can be seasonal [8]. Meantime some species are harvested from their natural habitats which leads to a huge issue in the sustainable growth of those plants [7].

Micropropagation techniques can be effectively used to overcome the issues in commercial aquatic plant production and propagation. Many studies have been conducted to determine the best conditions for each step of the micropropagation cycle, including explant sterilization, shoot and root induction, and acclimatization. The conditions required for each step may vary depending on the plant species and explant type used. In this review, we will identify micropropagation techniques introduced by different researchers for some aquatic plants like *Hemianthus callitrichoides*, *Rotala rotundifolia*, *Cryptocoryne* spp., *Anubias barteri* var. Nana, and *Limnophila aromatica*.

A. Hemianthus callitrichoides

Hemianthus callitrichoides 'Cuba' is one of the smallest aquarium plants in the world and belongs to the family Scrophulariaceae. When growing conditions are ideal, develops dense, carpet-like growth that spreads horizontally over the ground. In the tank, this growth pattern gives a lush and visually pleasing carpet impression.

- Explant Sterilization – Reference [9] tested for a better protocol to sterilize the explants of *H. callitrichoides*. As the sterilization of *H. callitrichoides*, the explants were washed, sterilized in double-distilled water, and divided into shoot clumps then surface-sterilized with 25% commercial bleach, NaOCl (Sodium hypochlorite), and 0.1% $HgCl_2$ (Mercury (II) chloride) for 5 minutes or 10% H_2O_2 (Hydrogen peroxide) for 45 minutes, followed by Mancozeb solution, and rinsed with distilled water and blotted on sterilized tissue paper. The shoot-clump explants were unable to be sterile on their surfaces with NaOCl and $HgCl_2$. They have concluded that for surface sterilization, H_2O_2 was more effective compared to the other treatments [9].

- Shoot Initiation- Similarly, according to [9], MS (Murashige and Skoog) media containing BAP (6-Benzylaminopurine) were the most suited and successful for the multiplication of shoots and their growth in a comparison of the tested other plant growth regulators [9]. For a wide range of *in vitro* applications, including callus induction, shoot regeneration, formation of roots, and somatic embryogenesis, MS media are frequently used. Another study showed that the full-strength MS medium and 1/2-strength POME (Palm Oil Mill Effluent) can give the highest growth index in *H. callitrichoides*. Then it has been demonstrated that 1/2 strength POME medium with liquid full-strength MS medium as a supplement is superior to full-strength MS medium and promotes the maximum growth of *H. callitrichoides* [10]. Sucrose serves as a fuel source for photomixotrophic metabolism during plant tissue culture, providing optimal growth. The shoots that were regenerated at greater sucrose concentrations were necrotic, limiting proliferation, and had a detrimental influence on chlorophyll synthesis [9]. The medium containing 4.5% sucrose showed the greatest weight gain, and the growing shoots were also more branched and vigorous [9].

- Root Initiation- For the root initiation, reference [9] used multiple regenerated shoots. For the *in vitro* rooting, an MS basal medium without PGR (Plant Growth Regulators) was used. On shoots that were grown on MS media lacking PGRs, rooting was encouraged [9]. In the media used to grow plants, agar is utilized to solidify and facilitate the healthy development of roots and the growth of culture. To grow larger clumps of *H. callitrichoides* with roots, the medium's solidification with 1 g/L agar was optimized [11].

- Acclimatization- For acclimation, the plantlets can be moved to aquariums with tap water and a foreground surface made of sand and gravel. Low light intensity during acclimation has an impact on plant development, however, *H. callitrichoides* is photophilic and cannot grow in low light levels [9].

B. Rotala rotundifolia

The perennial water plant *Rotala rotundifolia* has flexible stems that frequently branch to create low, creeping clusters. There are submerged and emergent variants of this species, and they differ in several ways as the submerged form features thin, lanceolate, sword-shaped leaves that are deeper green or

reddish, whereas the emergent form has fleshy, bright-green, rounded leaves [12].

• Explant Sterilization – To rid healthy plants of seaweed infestation, warm water can be used for washing. For *R. rotundifolia*, uninodal segments (0.5 cm) can be utilized. First, the pieces of rhizomes should be dipped in an ethanol solution (50% v/v) for 5-10 seconds, followed by washing in a NaOCl solution (1.5% w/v) for 20 minutes. They should be then rinsed in sterile distilled water and again dipped in the NaOCl solution (1.5% w/v) for 20 minutes. Finally, the *R. rotundifolia* uninodal segments can be cleaned using a 1.0% (w/v) concentration of NaOCl solution [13].

- Shoot Initiation- Reference [13] tested for a preferable shoot initiation medium for *R.rotundifila.* All leaves that exhibited no signs of bacterial or fungal contamination were grown on MS medium with 0.25, 0.50, 1.0, 1.50, and 2.0 mg/L BA (6-benzyladenine) fed with 3% sucrose and 0.65% agar solidified in magenta GA3 (Gibberellic Acid) containers for regeneration. A maximum number of shoots per explant was obtained from MS medium containing 2.00 mg/L BA and 0.20 mg/L GA3 [13].

- Acclimatization- Two different basal substrate layers should be placed at the bottom of the tank with 150 mm thickness. The first one should be composed of peat, fine clay, and sand (1:1:10 / v: v: v), and the second one should be like natural soil. All substrates should be autoclaved, and substrates should be maintained completely submerged. The rooted clumps (plantlets) can be directly acclimated in a PVC and glass tank (110 x 40 x 55 cm) that contains 160 L of deionized water and can be pre-conditioned at a 16-hour photoperiod and 25 W of artificial light. The aquarium's temperature should be kept between 20 and 25 °C [7].

C. Cryptocoryne spp.

With roughly 50–60 species of aquatic monocot plants, the genus *Cryptocoryne*, also known as the "water trumpet," is significant within the Araceae family of plants. Popular aquarium plants, the majority of *Cryptocoryne* species are native to Southeast Asia and flourish in both submerged and emerging states [14].

a. Cryptocoryne lucens

- Explant Sterilization – A proper explant sterilization protocol was found in reference [15]. After defoliating the aerial plants of *C. lucens*, 1.0 cm nodal explants were separated and cleaned in running tap water for one hour. The explants were then surface-sterilized by immersing them first in 50% (v/v) ethanol for one minute, then in 105% (w/v) NaOCl for 12 repetitions, followed by three rinses of five repetitions each in sterile distilled water [15].

- Shoot Initiation- Similarly shoot proliferation from different explant types (single and clustered triple-node shoot explants) was observed on Linsmaier and Skoog salts and vitamins medium (LS) with 20 µM BA and 0.5µM NAA (1-Naphthaleneacetic acid) solidified with 0.8% (w/v) agar. The total number of shoots produced per explant was significantly greater from triple-node explants. All shoots on BA-supplemented media rooted and developed dark green leaves with red margins and petioles [15].

- Rooting and Acclimatization- Four cells with dimensions of 6.1 × 3.8 × 6.0cm were used to transfer the rooting medium of triple-node micro-cuttings. To maintain high humidity, a clear vinyl propagation dome was used to cover the tray. The tray was soaked weekly with 20:13.9:3.7 (N: P: K) liquid fertilizer, containing 150 mg N/L. After 3 weeks, the propagation domes were progressively removed over 5 days to reduce humidity and help the plantlets acclimatize. The trays were then moved to a lightly shaded greenhouse with heating and cooling set temperatures of 18 and 29ºC, respectively. After 18 weeks, the plants had survived well and produced large leaves. Finally, the plants were acclimatized, and after 30 weeks, they started flowering [15].

b. Cryptocoryne beckettii

- Explant Sterilization – According to reference [14], *C. becketti* mother plants' shoot tips can be separated, and they are washed thoroughly under running tap water. After which it should be cleaned with sterile distilled water and soaked for 1 minute in 70% ethanol. They can be surface-sterilized for 15 minutes using a 200 mg/L (w/v) $HgCl_2$ solution, and then they should be thoroughly rinsed three times with sterile distilled water. Finally, they should be surface sterilized again for another 15 minutes using 15% Clorox solution (containing 5.3% NaOCl), 2–3 drops of Tween 20, and washed three times with sterile distilled water [14]. However, reference [7] showed that the rhizome of this plant also can be used as an explant. It has to be washed in warm water and separated into 1cm long pieces. The portions of rhizomes should be washed in ethanol solution (50% v/v) for 5 - 10 seconds and then dipped in NaOCl solution (1.5% w/v) for 20 minutes. Finally, the explants should be rinsed in sterile distilled water [7]. Similarly, reference [16] also used the rhizome of this plant as the explant. There *C. beckettii* rhizomes were washed well with soap and water and rinsed under running tap water for six hours. Then, overnight they were immersed in a fungicide [3% (v/v) 'thiophanate methyl' 70%'] solution. The rhizomes were washed again for one hour under running tap water, dipped in 70% (v/v) ethanol for 1 minute and 20% (v/v) NaOCl for 15 minutes. Finally, the explants were thoroughly washed five times with sterile distilled water [16].

- Shoot Initiation – According to the study conducted by reference [14], a higher shoot proliferation was formed after four weeks from MS medium supplemented with 0.5 mg/L BA + 30g/L sucrose and without agar [14]. For the shoot initiation of *C. becketti*, the explant was vertically positioned in LS medium, supplemented with NAA (0.5 mg /L) and BAP (2.0 mg/ L) in the study conducted by reference [7]. The cultures were transferred to a growing room and maintained at 22 ± 1°C and 16 hours of light photoperiod, under cool white fluorescent light. Then after six weeks, shoots were transferred to the higher multiplication medium of LS medium + 4 mg/ L of BAP [7]. In

the study conducted in reference [16], rhizomes were cut into 1cm length fragments and placed horizontally on full-strength MS medium with 3% sucrose. Finally, they were grown under white fluorescent light with a 16-hour photoperiod for shoot regeneration. After six weeks, they were grown on shoot multiplication media using MS medium + 5.0mg/L BAP + 0.1mg/L IAA (Indole-3-acetic acid) and obtained the highest mean number of shoots per shoot explant [16].

- Root Initiation- After four weeks in the study conducted in reference [14], root proliferation was observed in MS medium supplemented with 0.5 mg/L BA and 30 g/L sucrose, without agar [14]. Reference [7] found that transferring *C. becketti* plantlets to a low concentration of BAP (1mg /L) on LS medium with a higher number and length of roots resulted in better growth [7]. After eight weeks of culture on root initiation medium containing half-strength liquid MS supplemented with 0.1 mg/L NAA, plantlets in the study conducted in reference [16] achieved the highest mean root number, root length, and shoot height [16].

- Acclimatization- Eight-week-old rooted micropropagated *C. becketti* plantlets were thoroughly washed with running tap water before being acclimatized into plastic pots (20 x 20cm) in reference [14]. The acclimatization medium, which contained a mixture of compost and sand in a 1:1 ratio, was overlaid with tap water and placed under greenhouse conditions. The plantlets were exposed to a relative humidity of 80-90% and a temperature of 28 ± 2 ºC during the daytime and 24 ± 2ºC during the nighttime [14]. Reference [7] discovered that the rooted plantlets can be acclimated in an aquarium tank that has a size of 110 x 40 x 55 cm and contains 160 L of deionized filtered water. The tank should be maintained at a temperature between 20 and 25°C and have a pre-conditioned 16-hour photoperiod with 25W artificial light. At the bottom of the tank, a 150-mm layer of autoclaved basal substrates composed of peat, fine clay, and sand (in a ratio of 1:1:10 by volume) should be placed. After 15 days, all plantlets were found to have survived at 100% [7]. After eight weeks, the well-rooted plants in the reference [16] study were acclimated in clay pots with a mixture of sterile clay and sand in a 2:1 ratio. The plants were provided with sterile distilled water to a height of 1 cm. As a result, the plants survived very well [16].

D. Anubias barteri var. Nana

Anubias barteri var. Nana is a valuable aquatic plant that grows completely submerged in water and belongs to the Araceae family. It is widely used to enhance the well-being of ornamental fish and to create more lifelike aquarium decorations. Consequently, the demand for this plant is increasing globally daily [19].

- Explant Sterilization – In the study conducted by reference [19], the explant they used was stem nodes and for the surface sterilization, explants were rinsed in double distilled water after an hour of being cleaned under running tap water. After that, surfaces were disinfected for 10 minutes with a 25% commercial bleach solution and 15 minutes with a 75 mg /L antifungal solution (Mancozeb). After one more rinsing for 3 × 7 minutes with sterile double-distilled water, the explants were blotted on sterile tissue paper. Another study has shown that shoot tips can be used as the explant. There the plantlets were surface sterilized by using a 0.5% (w/v) $HgCl_2$ solution with two drops of Tween-20 emulsifier per 100 ml solution for a period of three minutes. To remove any residual disinfectant, the treated plantlets were rinsed three times with sterile distilled water. Afterward, the explants were subjected to another round of surface sterilization, this time with a 10% (v/v) dilution of commercial Clorox, which contains 5.25% NaOCl and 2 drops of Tween 20 per 100 mL solution. The explants were left in this solution for 15 minutes, followed by a 5% (v/v) Clorox solution for an additional 5 minutes. Finally, the explants were cleaned three times with sterile distilled water to complete the surface cleaning process [18].

- Shoot Initiation–Reference [17] found that the highest axillary shoot induction was observed on both half and full-strength MS media containing 0.25 and 0.50 mg /L GA3 respectively while the media were supplemented with a constant concentration of 0.10 mg/L BAP and 0.10 mg /L NAA. According to reference [18], in MS media supplemented with 3 mg/L BA, the greatest number of shoots per explant was achieved. The findings indicate that BA applied alone was critical for *A. barteri* var. Nana's activation of axillary bud development [18].

- Root Initiation–In the study conducted by reference [18] they revealed that on MS media free of plant growth regulators or kinetin alone, rooting in all regenerated shoots was encouraged [18].

- Acclimatization–The tap water-filled aquarium effectively acclimated the in vitro regenerated *A. barteri* plantlets without exhibiting any signs of mortality. Low photosynthetic photon flux (PPF) during acclimation, yet, had an impact on the growth of regenerated plants. Recall that *A. barteri* is a light-loving plant that might not thrive in a dark environment [17].

E. Limnophila aromatica

Also known as Rice paddy herb it is a tropical and stout aromatic herb 30-50 cm in height and belongs to the family Plantaginaceae [19]. This plant is native to Southeast Asia and typically grows in flooded rice fields and is commonly used as an aquarium plant [20].

- Explant Sterilization–To prepare *L. aromatica* plants for surface sterilization, they should be placed under running tap water for 15 minutes. First and second-node explants, which include the meristematic region, should then be treated with 20% and 30% NaOCl for 10 minutes, respectively [21]. If not, H_2O_2 can be used at a rate of 16% (v/v) for 10 minutes, followed by rinsing the plant tips with sterilized distilled water three times in five minutes. For propagation, sterilized twigs with 1-2 cm pieces and one to two nodes should be used and cultured on a suitable medium for 15 days. Reference [22] used shoot tips and young leaves as explants in their study [22].

- Shoot Initiation- In the study of reference [22], surface-sterilized shoot tip explants were inoculated on MS medium supplying 0.25-2.0 mg/L BA+ 0 and 0.25 mg/L NAA. Shoot regeneration frequency on the test was 100% of any culture [22]. Reference [21] demonstrated that callus could be generated from nodal explants by adding TDZ (Thidiazuron) and 2,4-D (2,4-Dichlorophenoxyacetic Acid) to the MS medium at a concentration of 0.25 mg/L each. The calli were then cultured in MS medium supplemented with 0.50 mg/L of kinetin. The MS nutritional medium was prepared using 3% sucrose [21].

- Root Initiation–According to reference [22], after culturing for a period of 8 weeks, when the isolated shoots were placed on a rooting medium containing 0.25-1.0 mg/L NAA, regardless of the concentration, rooting began within 8-10 days. After 4 weeks of culture, 100% rooting was observed. For both shoot induction and root initiation, the culture media must contain 30g/L (W/V) sucrose and 0.65% (W/V) agar at a pH of 5.8 [22].

- Acclimatization–In the study conducted by reference [23], it was found that the addition of water during the culture process had a positive impact on the growth and acclimatization of multiplied *Limnophila* plants. These plants are able to grow both in the presence and absence of water. After the addition of water, the morphology of the plant's leaves changed from aerial to submerged, with thinner and more linear leaves [23].

II. Conclusion

Micropropagation is a widely used asexual plant propagation technique in agriculture, particularly in the horticultural crop sector. The ornamental aquatic plant industry is experiencing a continuous increase in demand worldwide, being highly important for aquariums and water resource rehabilitation projects. However, traditional propagules pose several issues, making the use of advanced technologies like micropropagation a potential solution for commercial plant production. Research has focused on finding preferred protocols to culture ornamental aquatic plants, using different explants depending on the species. Choosing the best protocol is crucial for efficient plant production. As well as maintaining a healthy mother plant stock, breeding new varieties, and identifying potential hidden benefits through the fundamentals of plant tissue culture is important to the growth of this subject area.

References

[1] S.S. Bhojwani and P.K. Dantu, "Micropropagation," in *Plant Tissue Culture: An Introductory Text*, S.S. Bhojwani and P.K. Dantu, Eds. India: Springer, 2013, pp. 245-274.

[2] B. Kaviani, "Some useful information about micropropagation," *Journal of Ornamental Plants*, vol. 5(1), pp. 29-40, 2015.

[3] I. Iliev, A. Gajdoˇsova, G. Libiaková, S.M. Jain, "Plant micropropagation," in *Plant Cell Culture: Essential Methods,* M.R. Davey and P. Anthony, Eds. United Kingdom: John Wiley and Sons, 2010, pp. 1-23.

[4] B. Loberant and A. Altman, "Micropropagation of plants," in *Encyclopedia of Industrial Biotechnology: Bioprocess, Bioseparation, and Cell Technology,* M. C. Flickinger Ed. New York: Wiley, 2010, pp. 3499-3515.

[5] E.F. George, M.A. Hall, G.J.D. Klerk, "Micropropagation: uses and methods," in *Plant Propagation by Tissue Culture Volume 1. The Background* (3rd edition), E.F. George, M.A. Hall, G.J.D. Klerk Eds. Dordrecht, Netherlands: Springer, 2008, pp. 29-64.

[6] U.C.C. Jayarathna and S.P.S.D. Senadheera, "Current status of ornamental aquatic plants industry in Sri Lanka," *in Proceedings of the National Aquatic Resources Research and Development Agency (NARA)*, 2015, pp. 47-50.

[7] M. Micheli, A. De Gasperis, F. Prosperi, A. Standardi, "Micropropagation of three species of aquatic plants," *Agricoltura Mediterranea*, vol. 136(1), pp. 1-6, 2006.

[8] G. O. Dick, R.M. Smart, J.R. Snow, "Propagation and production of native aquatic plants," Aquatic Plant Control Research Program (APCRP) USA, September 2005.

[9] S. Barpete, S. F. Özcan, M. Aasim, S. Özcan, "*In vitro* high frequency regeneration through apical shoot proliferation of *Hemianthus callitrichoides* 'Cuba' - a multipurpose ornamental aquatic plant," *Turkish Journal of Biology*, vol. 39, pp. 493–500, 2015.

[10] Y. S. Ng, C. R. Lim, D. J. Chan, "Development of treated palm oil mill effluent (POME) culture medium for plant tissue culture of *Hemianthus callitrichoides*," *Journal of Environmental Chemical Engineering*, vol. 4(4), pp. 4890–4896, 2016.

[11] E. Özcan, H. H. Atar, S. A. Ali, M. Aasim, "Artificial Neural Network and decision tree–based models for prediction and validation of *in vitro* organogenesis of two hydrophytes—*Hemianthus callitrichoides* and *Riccia Fluitans*," *In Vitro Cellular and Developmental Biology – Plant*, vol. 59, pp. 547–562, 2023.

[12] C. Gu, F. Li, J. Xiao, S. Chu, S. Song, M.H. Wong, "A novel submerged *Rotala rotundifolia*, its growth characteristics and remediation potential for eutrophic waters," *Scientific Reports*, vol. 9(1), pp. 14855, 2019.

[13] M. Karatas, M. Aasim, M. Çiftçioğlu, "Adventitious shoot regeneration of round leaf toothcup- *Rotala Rotundifolia* [(Buch-Ham. ex Roxb) Koehne]," *The Journal of Animal and Plant Sciences*, vol. 24(3), pp. 838-842.

[14] C. Stanly, A. Bhatt, C.L. Keng, "An efficient *in vitro* plantlet regeneration of *Cryptocoryne wendtii* and *Cryptocoryne becketti* through shoot tip culture, *Acta Physiologiae Plantarum*, vol. 33, pp. 619-624, 2011.

[15] M. E. Kane, E.F. Gilman, M.A. Jenks, T.J. Sheehan, "Micropropagation of the aquatic plant *Cryptocoryne lucens*, *HortScience*, vol. 25(6), pp. 687-689, 1990.

[16] H.M.I. Herath, S. A. Krishnarajah, D. S. A. Wijesundara, "Micropropagation of two endemic threatened *Cryptocoryne* species of Sri Lanka," *Tropical Agricultural Research and Extension*, vol. 11, pp. 19-24, 2008.

[17] B. Surendra, M. Aasim, Ö. S. Fatih, Ö. Sebahattin, Effect of gibberellic acid on *in vitro* flowering from stem node explant of *Anubias barteri* var. Nana," *Research Journal of Biotechnology*, Vol. 14(9), 2019.

[18] K. Kanchanapoom, P. Chunui, K. Kanchanapoom, Micropropagation of *Anubias barteri* var. nana from shoot tip culture and the analysis of ploidy stability," *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, vol. 40(2), pp. 148-151, 2012.

[19] B. Yingngam, A. Brantner, M. Treichler, N. Brugger, A. Navabhatra, P. Nakonrat, "Optimization of the eco-friendly solvent-free microwave extraction of *Limnophila aromatica* essential oil," *Industrial Crops and Products*, vol. 165, pp. 113443, 2021.

[20] D. Gorai, S.K. Jash, R.K. Singh, A. Gangopadhyay, "Chemical and pharmacological aspects of *Limnophila aromatica* (Scrophulariaceae): an overview, *American Journal of Phytomedicine and Clinical Therapeutics*, vol. 2(3), pp. 348-356, 2014.

[21] M. Dogan, "Effect of salt stress on *in vitro* organogenesis from nodal explant of *Limnophila aromatica* (Lamk.) Merr. and *Bacopa monnieri* (L.) Wettst. and their physio-morphological and biochemical response," *Physiology and Molecular Biology of Plants*, vol. 26(4), pp. 803–816, 2020.

[22] M. Karataş and M. Aasim, "In vitro whole plant regeneration of the medical aquatic plant-*Limnophilla aromatic*," *Fresenius Environmental Bulletin*, vol. 24, pp. 1-4, 2016.

[23] T. Niki, and W. Amaki, "Micropropagation of ornamental aquatic plants, *Glossostigma, Microcarpaea*, and *Limnophila", Acta Horticulturae*, vol.1055, pp. 369-373, 2014.