Review Article

Micropropagation of Aquascaping Plants: A Review

ABSTRACT

Aquascaping is a creative arrangement of aquatic plants, rocks, stones, driftwood, and other elements within an aquarium to create visually pleasing underwater landscapes. It blends principles of design, horticulture, and aquaculture to either mimic natural environments or express artistic creativity. Aquascaping plants are aquatic plants selected and cultivated for their aesthetic appeal and ability to thrive underwater, enhancing the visual beauty of aquarium landscapes. These plants are chosen based on their capacity to flourish when submerged and their role in creating attractive underwater environments. Asaquascaping industry is gaining importance, rapid propagation of plants used in it plays pivotal role. Most of the plants are slow growers and their propagation is difficult rendering these plants very expensive.*In vitro* culturecan be a best alternative propagation method in propagation of aquascaping plants. In vitro propagation is highly relevant and beneficial in the context of aquascaping plants, where precise control over plant growth and health is crucial for creating and maintaining underwater landscapes (aquascapes).

Keywords: Aquascaping, Tissue culture, Aquariums, *In vitro* propagation

1. INTRODUCTION

Aquascaping involves the creative arrangement of aquatic plants, stones, rocks, or driftwood in a visually appealing manner within an aquarium[1]. It involves creating aesthetically pleasing creations under water using plants, rocks, substrates, and sometimes fish or other aquatic creatures. Aquascaping is a combination of art and horticulture to potray alluring and cordial aquatic scenes, often inspired by seascapes [2]. Aquascaping, a discipline within the aquarium hobby, has evolved through the contributions of various influential figures and groups over time. Takashi Amano, a notable figure from Japan (1954-2015), is widely recognized as a pioneer in modern aquascaping. He played a focal role in advancing the creation of naturalistic underwater landscapes, promoting layouts inspired by nature and emphasizing principles such as balance, harmony, and the integration of aquatic plants to replicate natural habitats. Tissue culture techniques have offered notable benefits in the efficient and accelerated production of plants [3]. Some plants commonly used in aquascaping include Java moss, Anubias, Cryptocoryne, Hygrophila, Ludwigia, Java fern, Amazon sword, Vallisneria, Pogostemon, and Rotala.

Tissue-cultured aquatic plants have seen a sharp increase in commercial interest recently. Tissue culture techniques have been successfully used to cultivate these plants, as numerous research have shown [4]. A key factor in the regeneration of shoots is the kind and quantity of growth regulators added to the tissue culture medium [5].One of the key advantages of tissue culture for aquascaping plants is the ability to produce pathogen-free, healthy plantlets [6]. This is particularly important in the aquarium setting, where the introduction of diseases or pests can have devastating effects on the entire ecosystem. Aquascaping plants often prefer tissue culture due to their sterile propagation conditions, which guarantee they are free from pests, diseases, and algae essential for maintaining a healthy aquarium environment. Furthermore, tissue culture plants are consistent in quality and appearance, ensuring uniform growth that aids aquascapers in achieving precise design aesthetics. Additionally, these plants tend to develop strong root systems, facilitating quicker adaptation to

underwater conditions compared to traditionally grown plants in pots or soil. In summary, tissue culture plants are valued for their reliability, cleanliness, and ability to thrive, making them a preferred choice for vibrant and thriving aquascaping displays. The demand is limited to specific markets and in five years (1990- 1995) the production in Holland increased from 417,000 to 1,204,000 plants, some of which propagated in vitro [7].

2. METHODOLOGY OF TISSUE CULTURE

2.1 Selection of Plant Material (Explant)

The tissue culture process for aquascaping plants typically involves several steps, Plant explants, such as leaves, stems, or meristems, are sterilized and positioned on a nutrient-rich media in an aseptic culture, which is the initial step. An explant is the term for the plant tissue used in culture.The effectiveness of the explant is influenced by its position on the plant, as well as the plant's age or developmental stage[8]. Explants that include shoot primordia, such as meristems, node buds, or shoot apices, are generally favored. Additionally, explants taken from younger or juvenile plants tend to be more successful.

Leaf explants are largely used in tissue culture as they have good potential to produce callus and shoots in many plants. Leaf explants of *Hygrophilapolysperma* were cultured on MS medium with varying concentrations of Kin or TDZ, either with or without 0.10 mg/L IBA. Direct adventitious shoot formation, occurring without intermediate callus formation, began at the leaf tips on media containing both Kin-IBA and TDZ-IBA. After one week of cultivation, distinct shoot buds appeared on the tips and edges of the leaf explants [9]. Microsorumpteropus was established successfully from leaf explant[10]. *Centella asiatica*was successfully cultured using leaf explants on MS medium along with BA and NAA and also obtained 81.6% regeneration using stem node explant [11].

Shoot tips are fastly dividing hence it is widely used in tissue culture. Regeneration potential of shoot tip explants of *A. heterophylla* was explored on MS medium containing 0.1 % (w/v) activated charcoal (AC) and supplemented with 6-benzylaminopurine (BAP) at different concentrations[12]. Regeneration of *Anubiasbarteri var. Nana* was accomplished via organogenesis using shoot tip cultures. Multiple shoots were successfully induced from the shoot tips when grown on a modified MS medium enriched with BA and kinetin. For micro shoot induction and leaflet production, nodal explants proved to be more effective than shoot tips in three aquatic species: *Lobelia cardinalis*, *Staurogyne repens*, and *Alternanthera reineckii*[13].Maximum number of shoots(25.33) were produced in *Hygrophilapolysperma*using cultured shoot tip as explant in liquid MS medium and 21.67 shoots from first nodal segment explant [14].

Another explant generally used in tissue culture of aquascaping plant is stem node. An ornamental aquatic plant *Ludwigiana* sp. had been successfully cultured from stem nodes containing single nodes with ± 1 cm[15].In *Hedychium coronarium*rhizome bud explants cultured in BA, KIN, and TDZ, recorded maximum number of 14.21 shoots per explant on medium enriched with 1.0 mg/L TDZ and 12.89 cm shoot length on medium provided with 1.0 mg/L BA. The plants were rooted on liquid ½ × MS medium containing 1.0 mg/L NAA with acclimatization [16].

2.2 Surface Sterilization

Sterilizing explants is a crucial step for effective *in vitro* micropropagation and can be achieved using various agents such as sodium hypochlorite, calcium hypochlorite, ethanol, mercuric chloride, hydrogen peroxide, and silver nitrate[17].For aquatic plants, effective sterilization is essential before micropropagation, as entire plants or their parts are subjected to sterilization and used as explants. It is vital to ensure that explants are sterilized without significant damage, making the choice of sterilizing agent critically important. Inadequate sterilization can impede micropropagation and lead to increased contamination. The choice of sterilizing agent is influenced by the morphological characteristics of the plant parts, such as tissue hardness or softness It is vital to ensure that explants

are sterilized without significant damage, making the choice of sterilizing agent critically important. Inadequate sterilization can impede micropropagation and lead to increased contamination [18].For aquatic plants, hydrogen peroxide (H_2O_2) is frequently used due to its lower damage potential and effectiveness, as seen in its application to species like Water Hyssop, Dwarf Hygro, and Coontail[9].For water lettuce, effective sterilization has been achieved using either 1.0-3.0% diluted commercial bleach or 8-24% diluted H_2O_2. For surface sterilization of aquatic plants, such as *Bacopa monniera*, sodium hypochlorite (NaOCl) has been shown to be more successful than mercuric chloride ($HgCl_2$) [19].The performance of H_2O_2 in sterilizing *H. callitrichoides* was superior. 37 explants, or 550 individual plants (82.22%), out of 45 shoot-clump explants treated with $H2O_2$ were successfully sterilized and established in vitro when cultivated on MS media without growth regulators [20].The best surface sterilization for *Staurogyne repens* was obtained by utilizing 0.1% mercuric chloride for 5 minutes; this produced about 60% of the explants free of contamination. On the other hand, 1% sodium hypochlorite and 5% chloramine B were either harmful to the plants or did not work to stop microbial growth.

2.3 Preparation of Culture Media

In order to support the growth and development of aquascaping plants in vitro, a nutrient-rich environment must be prepared for the tissue culture of the plants. The first step in the process is to choose a suitable base medium. Two options are Gamborg's B5 medium and Murashige and Skoog (MS), both of which offer a well-balanced combination of vital nutrients. The formulation contains calcium, magnesium, sulfur, and important macronutrients like potassium, phosphorus, and nitrogen. To guarantee complete nourishment, trace amounts of micronutrients including iron, manganese, zinc, and copper are also included. Next, to give energy for the developing plant tissues, vitamins, amino acids, and a carbohydrate source like sucrose are added to the medium.To guarantee complete nourishment, trace amounts of micronutrients including iron, manganese, zinc, and copper are also included. Next, to give energy for the developing plant tissues, vitamins, amino acids, and a carbohydrate source like sucrose are added to the medium. Vitamins, amino acids, and a supply of carbohydrates, such as sucrose, which gives the developing plant tissues energy.

The preparation involves dissolving these components in distilled water and adjusting the pH to approximately 5.8-6.0 using hydrochloric acid or sodium hydroxide. After achieving the desired pH, the medium is typically gelled with agar or Gelrite, which solidifies the medium and supports the plant tissues. The prepared medium is then sterilized by autoclaving at 121°C for 15-20 minutes to eliminate any potential contaminants. Once cooled, the sterile medium is poured into culture vessels, ready for inoculating the plant material. This carefully controlled environment is crucial for successful tissue culture, allowing aquascaping plants to grow and develop into healthy, viable specimens.

Use of liquid medium is also common in aquascaping plants culture. Liquid MS media is used in both rooting and shooting media.

2.4 Shoot Initiation

One of the most practical and economically successful techniques for propagating plants is micropropagation. The totipotency of plant cells—which enables them to regenerate entire plants in vitro—is the basis for this procedure. Since direct organogenesis is thought to be a more reliable method, it is frequently chosen for creating clonal plants that retain genetic consistency [21].This intricate method involves a combination of physical and chemical factors [22], and typically begins with the shoot or root meristem of the explant [23].The process is mainly regulated by the interaction of internal and external plant growth regulators.The genotype of the plant, the kind of explant, and its physiological state are some of the variables that affect these regulators' levels. Furthermore, the kind and concentration of plant growth regulators employed in the culture media are important factors in determining the course and outcome of organogenesis [24].The selection of appropriate shooting media is a critical factor in the success of micropropagation, as it can influence the growth, development, and genetic stability of the regenerated plants[25, 26].
BAP is widely used in aquascaping plants, BAP promotes shoot multiplication and can be optimized depending on the plant species. Most aquatic plants require standard BAP concentrations of 2 to 5 mg/L; however, these can be adjusted based on the responses of individual plants. In comparison to the higher 4 mg/L BAP concentration, the medium supplemented with 1 mg/L BAP for

Cryptocorynebeckettii, *Cryptocoryne lutea*, and *Rotala rotundifolia* induced satisfactory shoot proliferation and adventitious root formation, resulting in better root number and length. In contrast, the medium supplemented with 4 mg/L BAP produced higher rates of shoot multiplication but fewer roots in general [27].For shoot multiplication of *Anubiasbarteri var. nana petite*, the optimal medium was MS medium supplemented with 0.2 mg/L BAP, while lowering the BAP concentration from 0.2 mg/L proved effective for promoting shoot elongation [28].

Another cytokinin that encourages branch growth is kinetin; however, in some aquascaping plants, it is frequently less successful than BAP. All three aquatic plants *Llobelia cardinalis*, *Staurogyne repens*, and *Alternanthera reineckii* proliferated most quickly in the MS medium containing 2 mg/L kinetin[29].When administered with BAP or other cytokinins, kinetin's efficacy can be increased [30].*Anubiasbarteri* var. Nana was effectively regenerated by organogenesis with the use of shoot tip cultures. Cultured on a modified MS medium supplemented with BA and kinetin, several shoots emerged from the shoot tips. The ideal quantity of green shoots, averaging five shoots, was achieved using MS medium containing 3 mg/L BA. The best conditions for promoting the rooting of the regenerated shoots were MS medium supplemented with a single treatment of kinetin or without plant growth regulators. When the restored plants were put in field settings, they showed 100% acclimation and survival [31].
Combination of auxins and cytokinins are also used in shooting in case of some plants. Using single-node shoot explants on LS media supplemented with 20 µM BA and 0.5 µM NAA, the maximum shoot proliferation of *Cryptocorynelucens* was attained, with an average of 7.7 shoots per explant [32]. The best medium for *Linderniaantipoda* culture initiation and establishment was Half MS, which contained 1 mg/L of benzylaminopurine (BAP)[12].An alternative effective medium was 3.0 mg/L BAP, which yielded 2.40 ± 0.24 shoots per explant The highest shoot regeneration in *Lilaeopsisbrasiliensis* was observed with 1.5 mg/L NAA and 0.5mg/L BAP[33].For shoot induction, cytokinin and auxin combinations are also utilized. The combination of 1.0 mg/L NAA and 1.0 mg/L BAP improved shoot regeneration, yielding 3.60 ± 0.24 shoots per explant and 100% of explants regenerating new shoots within 60 days. For the Lysimachia species, MS medium enhanced with BAP and NAA 3 was the optimal shooting medium. The best medium for *L. christinae* and *L. rubinervis* contained 3.0-5.0 mg/l BAP and 0.1 mg/l NAA, resulting in the maximum number of shoots per explant (12.25–17.20) and 100% frequency of regeneration [34].

2.5 Root initiation

Root initiation is a crucial stage in the micropropagation process, which creates new plants in vitro from tiny tissue samples. Plant species conservation, genetic research, and mass propagation all make use of this technology. Rooting hormones, sometimes referred to as auxins or rooting regulators, are essential for the growth of roots from plant tissues in tissue culture. In vitro cultivated plant tissues, these hormones aid in the induction and development of roots. Since it promotes both the initiation and elongation of roots, indole-3-butyric acid (IBA) is frequently used to stimulate rooting in a variety of plant species. It is usually given at concentrations ranging from 0.1 to 5 mg/L and works especially well in woody plants and certain herbaceous species.The Murashige and Skoog (MS) nutrition medium supplemented with 0.25 mg/L Indole Acetic Acid (IAA) was shown to be the most efficient rooting medium for *Pogostemon erectus* (Dalzell) Kuntze. This effectively encouraged root formation and ensured the successful establishment of the plantlets [35].Massive formation of roots was observed in *Staurogyne repens* with use of a liquid MS media with addition of indole-3-acetic acid at concentration of 0.2 mg/l [36].

Indole-3-Butyric Acid (IBA) is widely used to induce rooting in various plant species; it facilitates both root initiation and elongation, with concentrations typically ranging from 0.1 to 5 mg/L, and is particularly effective for woody plants and some herbaceous species. The best medium for establishing dwarf hygrophila (Hygrophilapolysperma) was Murashige and Skoog (MS) medium enhanced with 0.20–1.00 mg/L of Indole-3-Butyric Acid (IBA). Bacopa monnieri in vitro cultivars were rooted in basal media; however, the addition of IBA greatly enhanced the rooting at 4.9µm levels [37]. The greatest quantity and longest roots of Limnophilaaromatica were found in MS media containing 0.25 mg/ml IBA [38].

In many plant species, Napthalene acetic acid (NAA) increases root production and encourages root initiation. It is frequently employed at concentrations between 0.1 and 5 mg/L and is frequently coupled with other hormones to improve rooting effectiveness. Although it can occasionally encourage the formation of roots, 2,4-Dichlorophenoxyacetic Acid (2, 4-D) is mainly employed as a synthetic auxin for callus induction and cell elongation. It is usually applied at concentrations between 0.1 and 2 mg/L and is more frequently used for callus induction than direct rooting. *Anubias Heterophylla* had more roots when auxin, α-Naphthalene acetic acid (NAA), or 2, 4-Dichlorophenoxy acetic acid (2, 4-D) were added [12].

2.6 Acclimatization

The effective transition of aquascaping plants grown in vitro from a controlled tissue culture environment to natural or semi-natural settings requires acclimatization. Generally speaking, plants created by tissue culture techniques are more expensive than those cultivated using conventional approaches. It takes a lot of time and work to move plantlets from growing containers to field settings. Depending on the species or even distinct kinds, there can be differences in the complexity and requirements of this transition. Plants frequently experience structural and physiological changes throughout the adaptation phase. This procedure guarantees the plantlets' survival and growth outside of the sterile culture environment while also assisting them in acclimating to external circumstances. Start by progressively exposing the plantlets to surroundings that are different from the sterile culture.
This can be achieved in a controlled atmosphere by gradually lowering the humidity and raising the light intensity. To reduce stress, place the plantlets in an enclosure or chamber with a high humidity level. A covered tray, a misting system, or a greenhouse with controlled humidity can all help achieve this. For a duration of one to two weeks, gradually reduce the humidity levels to facilitate the plantlets' adaptation to the reduced humidity of their surroundings. To prevent photo damage, progressively raise the light intensity, starting at lower light levels and working your way up to full light. Make sure you progressively adjust the temperature to the parameters of the intended growing environment. Check the plantlets frequently for indications of illness or stress. Keep an eye out for signs like withering, becoming yellow, or stunted growth. Make necessary adjustments to humidity, light, and temperature based on the observations of plant health. This disparity affects how quickly water evaporates from the leaves.

In the micropropagation process, hardening media is essential, especially for acclimating in vitro produced plantlets to ex vitro environments. Bridging the gap between the regulated in vitro culture and the natural or semi-natural circumstances in which the plants will ultimately be grown is the main objective of hardening media. Plants used in aquascaping are frequently produced in nutrient-rich, controlled environments, so reintroducing them to natural or semi-natural settings can be challenging. In order to help these aquatic or semi-aquatic species adjust from in vitro circumstances to aquarium or terrestrial settings, hardening media for aquascaping plants must satisfy their specific needs. Various hardening medium are used to help aquascaping plants that are grown in vitro acclimate to aquarium or terrestrial habitats more easily. Plant growth is supported by aqua soil, which also supplies vital nutrients. Good drainage and stability are guaranteed by gravel; well-liked varieties include SeachemFlourite Gravel and CaribSea Eco-Complete. With items like SeachemFlourite Sand and Pool Filter Sand, sand provides a natural appearance while supporting fragile roots. Nutrient retention is improved by clay-based substrates like ADA Aqua Soil and Fluval Plant and Shrimp Stratum. Perlite and vermiculite enhance aeration and moisture retention, whereas peat moss contributes moisture and acidity. Excellent drainage is provided by hydroton, and a well-balanced structure is guaranteed by soil-based mixes. Customized growth conditions can be established to fit the needs of individual plants by combining different substrates. Nymphoides indica was effectively acclimated by using clay pots with a 3:1 mixture of peat moss and decomposed cow manure. These pots were then submerged in culture tanks 10 cm below the water's surface and exposed to full sunlight [39]. For *Cunilagalioides*, rooted plantlets were acclimated by moving them into plastic chambers containing a sterilized sand-soil mixture (1:1). Initially covered with a plastic cap, the cap was gradually removed over a period of two weeks before the plants were transitioned to a greenhouse and later to outdoor conditions[40]. Hardening of *Bacopa monnieri* plantlets is done by by placing them in polybags with a 1:1:1 mixture of sand, farmyard manure, and soil, irrigating them with half-strength MS medium in a mist chamber for three weeks, then transitioning to an open shade

house with tap water irrigation for one month before field transfer[41]. *Ludwigiana repens* plantlets were effectively adapted in aquarium tanks with a 100% success rate in a study by [42]. In fiber tanks housed within a net house, *Bacopa caroliniana*, *Anubias minima*, *Aponogeton ulvaceus*, *Rotala rotundifolia*, and *Nymphoidescristatata* were successfully hardened utilizing a substrate mixture of pond soil and coir fibers[43]. A soil mix similar to natural conditions (peat:clay in a 1:1:10 ratio) produced superior shoot and root growth for acclimating *Cryptocorynebecketti*, *Cryptocoryne lutea*, and *Rotala rotundifolia* in aquarium tanks than the commercial Compo Cactea© mix [26].

3. CHALLENGES IN MICROPROPAGATION OF AQUASCAPING PLANTS

Aquascaping plant micropropagation has a number of difficulties, such as the requirement for specialized nutrient formulations to promote healthy plant development and the necessity for exact control over growth conditions. Plant responses to growth regulators and medium compositions are inherently variable, which can make successful plant regeneration more difficult. Additionally, because there is a significant chance of contamination from microbial sources, preserving sterility throughout the culture phase is essential yet challenging. Acclimatization problems are frequently the consequence of moving from in vitro to ex vitro settings; plants may find it difficult to adjust to their new surroundings, which can result in high mortality rates. Additionally, it can be expensive and hard to optimize techniques for various plant species, necessitating a great deal of trial and error. To overcome these obstacles, careful attention to detail, thorough testing, and adaptation of protocols to the specific needs of each aquascaping plant species.

Micropropagation has become a crucial technique for the efficient and sustainable cultivation of ornamental aquatic plants, offering significant benefits such as the production of numerous uniform, disease-free specimens and the capacity for rapid multiplication. However, this method also presents several challenges, including the necessity for precise control over growth conditions, the risk of contamination, and the complexity of tailoring protocols to different species. Effective micropropagation demands a thorough understanding of plant physiology along with a customized approach to growth media, regulators, and acclimatization strategies. Ongoing advancements in this field are improving the efficiency and success rates of plant propagation, which supports more sustainable practices in aquascaping and helps conserve valuable aquatic species. Future research should aim to address existing challenges, refine protocols, and explore new techniques to further enhance the micropropagation of aquascaping plants.

4. Conclusion

Micropropagation has emerged as a key method for the productive and sustainable development of ornamental aquatic plants. It has a number of advantages, including the ability to multiply quickly and produce a large number of uniform, disease-free specimens. Nevertheless, there are a number of drawbacks to this approach as well, such as the need for exact control over growing conditions, the possibility of contamination, and the difficulty of customizing protocols for various species. Effective micropropagation demands a thorough understanding of plant physiology along with a customized approach to growth media, regulators, and acclimatization strategies. Ongoing advancements in this field are improving the efficiency and success rates of plant propagation, which supports more sustainable practices in aquascaping and helps conserve valuable aquatic species. Future research should aim to address existing challenges, refine protocols, and explore new techniques to further enhance the micropropagation of aquascaping plants.

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