In Vitro Propagation of Aquatic Plants: A Review.

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ABSTRACT

In this exploration of *in vitro* propagation techniques, I delve into the latest research surrounding a diverse array of aquatic and medicinal plant species, including *Anubias barteri*, *Ceratophyllum demersum*, *Ludwigia repens, Cyperus papyrus,* and *Azolla species*. The papers examined in this study present methodologies and findings related to plant regeneration in these species, with a particular focus on *in vitro* micro-propagation techniques. The purpose of this exploration is to provide an in-depth look at these experiments, the innovative techniques employed, and their potential applications in the realms of clonal plant production, medicinal properties, and biofuel development. These compression of studies unravel the ever-evolving landscape of *in vitro* cultivation for aquatic and medicinal plants, as it useful for embarking on an intellectual journey to discover the promising horizons of plant tissue culture.

Keywords: *In vitro*, aquatic, medicinal, aquascape.

INTRODUCTION

Aquatic plants do not need a rigid trunk because they are supported by buoyancy in the water. Buoyancy devices such as gas-filled stomata and intercellular spaces maintain them, allowing them to grow towards the water surface and receive enough sunlight for photosynthesis. Aquatic plants play a pivotal role in various commercial sectors. They are highly sought after for aqua scaping, adding aesthetic charm to aquariums and water gardens. Additionally, some aquatic plants are a dietary staple, while others possess medicinal properties and contribute to the herbal medicine industry. Aquatic plants also aid in environmental remediation, helping purify polluted waters through phytoremediation. They are promising candidates for biofuel production due to their efficient biomass conversion. Beyond their commercial value, these plants are subjects of scientific research and conservation efforts, offering insights into ecological relationships and wetland preservation. They enhance landscapes, making them popular choices for decorative features in parks, gardens, and commercial properties. The commercial importance of aquatic plants is wide-ranging, spanning from ornamental and functional uses to environmental conservation and innovative industries.

Some examples of aquatic plants and their Uses: [33]

1. *Nelumbo lutea*: Native Americans have used the seeds and tubers of the plant as a source of food, and the leaves and flowers of the plant serve as a home and shelter for various aquatic animals.

2. *Alternanthera philoxeroides*: Alligator weed has some advantages like erosion control and water purification, but its fast growth can cause the displacement of native plants and disturbance of aquatic ecosystems.

3. *Eichhornia crassipes*: Water hyacinth, which is common in ponds, canals, rivers, and ditches, also provides benefits such as water purification and habitat for aquatic creatures.

4. *Rorippa amphibian*: This adaptable plant improves the beauty of aquatic environments while offering food and shelter to aquatic creatures.

5. *Ceratophyllum demersum*: This submerged plant directly absorbs nutrients from the water, which helps preserve the water's quality, because it doesn't have true roots.

6. *Lemna minoractually*: Serves a crucial purpose in maintaining the ecological balance and diversity of native aquatic habitats. It also provides necessary shelter for various aquatic species.

7. *Typha latifoli:* Used for centuries in various applications, such as making mats and baskets, and they also provide habitat for numerous aquatic creatures.

8. *Pontederia cordata*: Pickerelweed not only provides essential cover and breeding grounds for various aquatic organisms but also plays a pivotal role in improving water quality by absorbing excess nutrients, making it an indispensable component of many wetland ecosystems.

9. *Acorus calamus*: Sweet flag serves a practical purpose by preventing soil erosion in these water-edge locations and providing shelter for various aquatic fauna.

10. *Vallisneria Americana*: Plays an important role in stabilizing sediments, preventing shoreline erosion, and filtering excess nutrients to contribute to water clarity.

In vitro micropropagation has the potential to revolutionize many industries, including the creation of cloned plants, the development of innovative herbal therapies, and the sustainable production of biofuels. The complexity of the medium and the complexity of adaptation are important elements of this scientific journey. This review reveals the future of aquatic and medicinal plant production and captures the relentless pursuit of scientific progress. By analyzing the symbiotic interaction between culture medium and acclimation in the context of *in vitro* micropropagation, I aim to literate modified methods for increased multiplication and research in aquatic plants.

In Vitro Establishment of Aquatic Plants

The *in vitro* establishment of aquatic plants is a meticulous and pivotal process that unfolds in several key stages. It commences with the careful selection of suitable explants, such as shoot tips or nodal segments. These chosen materials are subjected to rigorous surface sterilization to the establishment of axenic cultures. The explants are then inoculated on a specially formulated culture medium containing precise nutrient combinations and growth regulators

to the unique needs of the specific aquatic plant species. Within this controlled environment, shoots are induced to regenerate under manipulated growth conditions. Regular sub-culturing is essential to ensure their continued healthy growth.

A later challenge is adaptation, where plants gradually move to the specific conditions of the target aquatic environment, including water quality, temperature, and lighting. Once these plants have successfully adapted in the laboratory, they are ready to be transferred to natural aquatic ecosystems/environments or aquariums, completing the cycle. This technique is crucial for the conservation, research, and commercial propagation of aquatic plants, especially those that are rare, endangered, or valued in aquaculture.

In Vitro Establishment: Problems

Several issues are affecting the health and quality of the plants. **Firstly,** there is a problem with certain plant tissues which rapidly turn brown when they are damaged or injured and exposed to the air. This browning may indicate some underlying physiological or environmental issues. **Secondly**, many former explants exhibit diverse fungal and bacterial activities, which can be detrimental to their overall health and growth. Explants from various locations and ecosystems are coated with a layer of microbes commonly found on surfaces, potentially contributing to contamination and plant stress. Several explants acquired from a neighborhood aquarium are contaminated with surface pollutants and dust. **Thirdly**, numerous leaves from former plants have an uppermost layer of scale that has dried out or perished, which can impact their ability to photosynthesize. **Finally,** ensuring genetic uniformity during propagation is a challenge. The creation of undesirable phenotypes can hinder the regeneration potential of specific plant varieties when genetic consistency is not guaranteed. These issues collectively pose challenges to the overall well-being of the aquatic plants and require careful attention and management.

Solutions for *in vitro* establishment, growth, and development:

1. To reduce the browning of explants, they are immersed in an in-antioxidant solution. Solutions of 0.2% Sodium diethyldithiocarbamate (SDDC) with benzyl adenine (BA).
2. Choice of explants: most of the time upper sections of the shoot twigs encompassing the apical meristem through the fourth axillary bud were isolated. This meristem gives axenic culture establishment.
3. Pre-surface sterilization, to reduce the microbial load of explants: Explants are washed under tap water for 30 minutes to remove the surface dust and to decrease contaminants. Followed by treatment with a solution of contact and systemic bactericide and fungicide (each-100mg/l) for 30 min.
4. To obtain axenic cultures explants treated with mercuric chloride ($HgCl_2$) and/or commercial bleach containing sodium hypochlorite to prevent the development of undesired fungal and bacterial contamination that would damage the quality and regeneration ability of the tissues.

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Table 1: Globally findings of *in vitro* kinds of literature on aquatic plants

Sr. No.	Variety	Explants	Surface sterilization	Medium composition			Incubations condition	Best morphogenic responses recorded in medium supplemented with	References
				Medium Used	PGR	Solidifying Agent			
1	*Anubias barteri* var. undulata	Shoot tips	Sodium hypochlorite	MS media	0.3 BA, 0.01 TDZ and 0.1 NAA	Sigma Type A agar	25-27 °C	0.3 BA, 0.01 TDZ and 0.1 NAA.	[1]
2	*Anubias barterii* Var. Nana petite	Apical buds	0.1% $HgCl_2$ and 5.25% NaOCl	MS media	0.1 to 0.3 mg/l BAP, 0.1 to 0.3 mg/l kinetin and 0.1 to 0.3 mg/l BAP 0.1mg/l NAA.	Agar	25 ± 2 °C	Roots produced on BAP 0.1mg/l NAA.	[2]
3	*Azolla rubra*	Shoot tips	0.05 % of $HgCl_2$	MS medium	NAA (2.69-10.74µM) without or with BA (0.22-4.44 µM)/Kn (0.23-4.65 µM) did not induce callus, 2,4-D (2.26-9.05 µM) for induced callus, 4.52 µM 2,4-D for induction and proliferation of callus.	Agar	-	2.26 µM 2,4-D and 6.2 µM 6-BAP, 2.26 µM 2,4-D and 6.6 µM 6-BAP	[3]
4	*Ceratophyllum demersum* L.	Shoot tips	-	MS medium	TDZ (0.05- 0.80 mg/L). BAP (0.25–1.25 mg/L).	Agar	Culture room at 24^0c	0.10 mg/L TDZ MS containing 0.40 mg/L TDZ. Shoot tip explant cultured on MS 0.40 mg/L TDZ was most effective	[4]
5	*Bacopa monnieri* L.	Shoots	40% diluted H_2O_2 for 10 min	MS medium	1.5 mg/l BA + 0.1 mg/l NAA + 0.1 mg/l GA_3	Agar	-	2.0 $mg.l^{-1}$ BA and 0.2 $mg.l^{-1}$ TDZ.	[5]

6	*Bacopa monnieri* L. Pennel	Shoots	40% diluted H_2O_2 for 10 min	MS medium	4.44 µmol/L BA and 0.54 µmol/L NAA.	Agar	5.8 ± 0.1	2.0 mg/l BAP and 0.2 mg/l NAA.	[6]
7	*Ludwiga repens*	Shoots	20 min in 30% Axion 1-2 drops	MS medium	0.1-0.3 mg dm-3 BAP, 0.1 mg dm-3 NAA or 0.05-0.15 mg TDZ and 0.1 mg dm-3 NAA	Agar	28 + 2°C	For meristems 0.05 mg/l TDZ and 0.1 mg /l NAA. 0.05 mg dm-3 TDZ and 0.1 mg dm-3 NAA or 0.3 mg dm-3 BAP and 0.1 mg dm-3 NAA.	[7]
8	*Pogostemon erectus* Dalzell Kuntz	Shoot tips	10% H_2O_2 (Merk) for 10 min	MS medium	0.25-1.25 mg/L KIN 0.25 mg/L NAA, IAA (0.25-1.00 mg/L	Agar	16 h light photoperiod (5000 lux)	KIN (0.25-1.25 mg/L) singly or combination NAA 0.25 mg/L.	[8]
9	Americn lotus *Nelumbo lutea* (Willd.)	Immature flowers	surface sterilized by successive immersion in 50% ethanol for 1 min, and 2.6% NaOCl for 12 min	MS medium	290 x m GA3 (100 mg-liter-1). 0.38 pM ABA	Agar	-	Our results indicate that American lotus can be easily established and cultured *in vitro* from excised embryos.	[9]
10	Umbrella plant (CYPERUS ALTERNIFOLIUS*)*	Nodes	10% commercial bleach for 15 min	MS Medium	-	Agar	28 °C	MS supplemented 30g/l sucrose, 8g/l Agar and 0 ,0.05 , 0.1 or 0.2 Mm mannitol.	[10]
11	*Persicaa amphiba*	Briefly, air-dried, and powdered abovegrou nd plant material	80% ethanol	MS media	0.5 mg/mL (3-4,5-dimethylthiazol-2-yl)-2, 5- MTT	Agar	DMEM containing 10% fetal bovine serum, 1% penicillin/str eptomycin, and 2 mM L glutamine), at 37°C, 5% CO_2, and 100% humidity.	The ∑FIC value, calculated for the IC_{25} and IC_{50} concentrations, showed additive interactions between P. amphibia and Dox for both the applied values (1.16 and 1.22 for IC_{25} and IC_{50}, respectively).	[11]

12	*Pogostn erectus* (Dalzell) Kuntze	Shoot tips and nodals	H_2O_2	MS media	0.25 mg/L IAA 2,4-D)	Agar	24°C	0.10 mg/L TDZ + 0.10 mg/L 2,4-D in the shoot explant, 0.10 mg/L TDZ + 0.10 mg/L 2,4-D, followed by in the MS supplemented with 0.20 mg/L TDZ + 0.10 mg/L 2,4-D (1.55 cm).	[12]
13	TULIP (*Tulipa gesneria* L.)	Apical and lateral buds, Disc stem, Fleshy leaf segment, and disc stem + apical buds	1.05% NaOCl + 2-3 drops of Tween-20 for 15 minutes. And $HgCl_2$ solution with 2-3 drops of Tween-20 for 15 minutes.	MS media	4.43 g L-1 MS salts, 100 mg L-1 Myo-inositol, 10 mg L-1 nicotinic acid, 0.1 mg L-1 Pyridoxine, 10 mg L-1 Thiamine-HC, 1 mg L-1 Glycine, 170 mg L-1 Na_2HPO_4, 40 mg L-1 adenine sulphate, 1 g L-1 PVP and 30 g L-1 sucrose. Cytokinin BA 0.5, 1.0, 1.5, 2.0 and 2.5 mg. 0.5 mg. L-1 NAA	Agar	25 ± 2°C	MS +1.5 mg L-1 BA to the bulb formation, which reached 72.17% and 2.67 bulbs per shoot. shoots cultured 1.0 mg L-1 BA were bulb weight.	[13]
14	*Lindera antipoda* L.	Rapid shoots	NaOCl 4% Chlorine 150 mg/l Bavistin BASF, and 0.03 % Ampicillin	MS medium	1 mg/L BAP and 0.2 mg/L NAA	Agar	25 ± 2 °C.	shoot formation. 1mg/l BAP and 0.2mg/l NAA. With 1mg/l BAP & 0.2 mg/l NAA (BM_3)	[14]
15	*Crinum malabaricm*	Shoots	0.1% Tween-20 solution for 20 min	MS medium	2.0 mg L−1 6-BAP 1.0 mg L^{-1} IBA	Agar	25 ± 2 °C.	IBA in rooting of the shoots and formation of bulblets in the member of the family Amaryl-lidaceae. None of the	[15]

								previous reports agreed with the present. findings on induction of roots by use of IBA in the Amayllidaceae members.	
16	*Sagittaa latifolia* Ecotyps	Shoot tips	0.56mM myo-inositol,1.2µM thiamine, 87.6 mM sucrose	MS medium	0.25mg/l^1 BA	Agar	-	Indexed shoot culture clonally multiplies each ecotype by ribosome production in BM supplemented with 0.25 mg l^1(BA) and solidified with 7 liter^1 TC Agar ™.	[16]
17	*Zantedescha aethiopica* (L)	Rhizomes and leafs	decontaminated with 70% ethanol for 60 s fol- y lowed by a solution of 1% Benlate for 30 min	MS medium	6-BAp and IBA	Agar	25 ± 2°C	BA and a single concentration of IBA. MS supplemented 4 mg 1 BA and 1 mg IIBA produced axillary shoots.	[17]
18	*Lobelia cardinalis* L	Shoot tips	4% Clorox 5.25 % NaOCl again surface sterilized for another 2% Clorox for 5min	MS medium	BAP; 1.0-3.0 mg L and NAA; 1.0-3.0 mg L^1	Agar	26 ± 2°C	maximum shoot 1.0 mg L 1 BAP + NAA; 1.0-3.0 mg L^1 after 60 d of culture	[18]
19	Lotus (*Nelumo nucifer*)	Buds	75% ethyl alcohol for 30 s, 0.1% $HgCl_2$ for 7 min	MS basal medium	6-BA , NAA , IBA , 2,4-D	agar (sigma)	27 ± 2°C	The maximum response was obtained with 2.22 µM 6-BA well-developed roots 0.54 µM NAA and 30 g/L sucrose.	[19]
20	Lotus (*Nelumo nucifer*)	Buds from rhizomes	75 % ethanol for 1min	MS medium	4.44 µM BA + 0.54 µM NAA	Agar	25 and 30 °C	NAA 2.69 to 5.37 µM, and root formation then decreased when NAA higher than 8.06 µM. The supplement of 0.44 µM BA.	[20]

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21	Americn Lotus	Fruits	50% ethanol,2.6% sodium hypochlorite	MS medium	N-1H BA, 2-methyl-4- (l/f-BAP, zeatin, (la, 2p, 4aa, 4bp, 10p)-2,4a, 7-trihy- droxy-l-methyl-8-methylenegibb-3-ene-l, 10-dicarboxylic acid l,4a-GA3, [S- (Z,E)]-[5-(l-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-l-yl)-3-methyl-2,4-ABA.	Agar	25 ± 2°C	Rhizome growth inhibited 0.38 pM ABA . Chemical names used: N-lH BA, 2-methyl-4, 2 zeatin, (la, 2p, 4aa, 4bp, 10p)-2,4a, 7-trihydroxy-l-methyl-8-methylenegibb-3-ene-l, 10-dicarboxylic acid l,4a-GA3, [S-(Z,E)]-[5-(l-hydroxy-2,6,6-trimethyl-4-oxo-2-cyclohexen-l-yl)-3-methyl-2, 4- ABA.	[21]
22	*Marsilea quadrifolia*	Petioles and leaves	ethylic alcohol (70%)	MS Medium	0.57 µM IAA , 0.53 µM NAA	Agar	24 ± 2 ^{0}C 16hr	MS ½ and 4 g/l agar MS + 0.57 µM IAA, MS + 0.53 µM NAA, Knopp liquid Knopp with 4 g/l agar, Knopp with 8 g/l agar for rooting and shooting.	[22]
23	*Aponogeton ulvaceus* Baker	Immature tubers	70 % ethanol for 30 s 0.4 % NaOCl for 20 min.	MS medium	0, 1, 2, and 3 mg/L of BAP, 3 mg/L NAA	Agar	25 ± 1 °C	(0-3 mg/L) of BAP and NAA for callus induction, 1 mg/L BAP and 3 mg/L NAA, and 2 mg/L BAP and 3 mg/L NAA. For organogenesis.	[23]
24	Myriophylm species[1]	Aerial – leafs	1.05% NaOCl containing 0.01% Tween-20 for 12min	MS medium	-	Agar	25 ± 2 °C	Rapid shoot regeneration of M. heterophyllum was sustained when explants (ca 8.0 mm) from the original shoot masses were subcultured onto fresh SMM.	[24]

25	*Ceratophyum demersum* L.	Nodes	5% H_2O_2	MS medium	Shoot 0.10-0.80 mg/L GA_3 and 0.40 mg/L TDZ , root 0.80 mg/L GA_3.	Agar	23±1°C	shoots formed. 0.40 mg/L TDZ was recorded as the most effective explant. 0.10-0.80 mg/L GA_3 to GA_3 on shoot elongation.	[25]
26	*Pogostemon erectus*	Shoots	10% H_2O_2	MS medium	1NAA. IAA	Agar	Incubated under 16 h light photoperiod (5000 lux)	0.25 mg/L 1NAA. IAA (0.25-1.00 mg/L) for root and shoot formation.	[26]
27	*Limnophilla aromatic*	Nodes	H_2O_2 at a rate of 16% for 10 min	MS medium	2.0mg/L BAP 0.1mg/L IAA 1.5mg/L IBA	Agar	Incubated in culture room at 25+2°C	Maximum response in terms of number of shoots MS medium supplemented with 2.0mg/L BAP.	[27]
28	*Alternanthera philoxeroides*	Roots	90% Ethanol 0.1%$HgCl_2$	MS medium	BAP (0,1,2 and 3mg/L NAA (0,1,2 and 3mg/L)	Phyta-ager	Immature tuber tissue with meristems	The morphogenetic response of callus to form roots of different BAP and NAA Concentration.	[28]
29	Ceratophyllum demersum L.	shoots	sterilized accordingly to Karatas et al. (2014)	MS medium	(BAP) (0.25, 0.50, 0.75, 1.00 and 1.25 mg/l) and 0.25 mg/l 1-NAA in Magenta GA7	Agar	24°C	Highest shoot formation MS medium fortified with 0.25 mg/l BAP + 0.25 mg/l NAA.	[29]
30	*Cryptocoryne Lucens*	Shoot tips	50% ethanol for 1 min and 105% Na_2HPO_4	LS medium	0.5 µM NAA	Agar	25 ± 3^0C	Average production of »150 singlenode microcuttings per culture vessel can be generated per 35-day culture cycle on LS plus 20 µM BA and 0.5 µM NAA.	[30]
31	*Cryptocorynelutea, Rotala totundifola* and *cryptoryne*	Shoots	(50%) ethanol	Linsmar and skoog	NAA (0.5mg l^{-1})	Agar		In all species ,the medium containing 4	[31]

	becketti		(1.5%) Na_2HPO_4		BAP (2.0 mg l^{-1})		--	mg l -1 of BAP resulted in higher multiplication rates as clearly shown.	
32	Glossostima, Microcarpaea and Llimnophila	Shoot tips	-	MS medium	-	Agar	23 ± 1^0C	All the species showed the best growth on the ½ MS The best concentration for the normal growth is also 20 g L-1 in M.minima and Limnophila.	[32]

Conclusion:

In *in vitro* establishment of aquatic plants presents a promising avenue for addressing the challenges associated with their commercial exploitation. As we have explored, the propagation and cultivation of aquatic plants in controlled laboratory environments offer innovative solutions for the mass production of species with ornamental, medicinal, and ecological significance. To overcome these obstacles and to scale up production for commercial value it is important to understand the levels of plant growth regulators of various species because it varies depending from species to species and plant to plant. Endogenous and exogenously supplied plant growth regulator plays an important role during the regeneration of plantlets. To increase rooting response, it is important to lower down endogenous level of cytokinin, for this one passage is carried out on a lower concentration of auxins or on a growth regulator-free medium. This review paper study describes a useful protocol for true-to-type mass propagation of aquatic plants which will contribute to meeting its increasing.

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