Tissue culture for mass production of aquatic plant species

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The aquatic plant export industry of Sri Lanka has been developing rapidly. This industry requires a continuous supply of high quality plants on a large scale. On the other hand, the long term interests of both industry and the ecosystem make it necessary to prevent wild and unchecked collection and to conserve these valuable species. This article discusses the mass propagation of aquatic plants for the export market while conserving the aquatic eco-system.

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Introduction

Natural fresh water ecosystems are unique in their diversity and vulnerable like all other tropical rain-forest ecosystems. From an ecological point of view, these aquatic environments possess unfathomable value and are aesthetically unmatched by any artificial invention. Modern man, trapped in an artificial world, tries consistently to surround himself with even a small part of this natural beauty. An obvious example is an aquarium, decorated so as to reflect the wonders of the natural environment.

Hundreds of fascinating and attractive plants are grown in aquaria for beauty and to maintain the quality of water. In addition, aquatic plants provide food, shade, shelter and breeding places for many life forms including fish. High demand, mainly from the developed countries, for aquatic plants have spawned large industries in both developed and developing countries.

International demand for aquatic plants has shown a steady increase in the past few years. In 1997, nearly 4.2 million aquatic plants were exported from Sri Lanka. That almost doubled in 1998 with the export of about 8 million plants. The value of exports of all ornamental plants and cut flowers from Sri Lanka has been consistently increasing since 1980. In 1980, exports valued only Rs. 1.68 million, but this figure increased to 191 million in 1990. In 1996, it increased to Rs. 454 million and in 1999, it reached a high of Rs. 573 million.

Most of this income has been from exports of live plants, including considerable amounts of aquatic plants, branches, leaves, etc. In response to the high demand in European countries, five large aquatic plant industries have emerged in Sri Lanka in recent years. With their need to supply unprecedented quantities of plants for the export market, these industries have begun exerting tremendous pressure on the

natural aquatic ecosystems of the island nation. This, in turn, has made it imperative and urgent to devise safe systems that, on the one hand, can supply plants rapidly at a large scale to meet export demand and, on the other, can protect the plant species and associated organisms in the ecosystem.

Aquatic ornamental plants

Aquatic plants are simply defined as all seed-bearing plants whose photosynthetically active parts are permanently, or at least for several months each year, submerged in water or float on the water surface. These plants show successful growth in water and have the ability to transport oxygen down to their roots and rhizomes from aerial or photosynthetic tissues.

Some water plants have evolved an advanced system which allows a mass transport of air. For example, water lilies (*nympheae*) have a system that ventilates the roots and rhizomes by means of air flow when the leaves contact air. Submerged plants have loosely arranged parenchyma cells which allow the diffusion of oxygen from green tissues down to roots and rhizomes. Most water plants are able to carry out metabolism based on fermentation. The availability of gaseous carbon dioxide is also a critical factor when growing under submerged conditions.

Some aquatic plants have evolved advanced strategies to overcome carbon limitation in their habitats. The most important adaptation is the ability to utilize bicarbonates (HCO_3) which are present at higher concentrations in alkaline water. In most modern aquaria, a system for CO_2 enrichment is available to overcome potential limitations in CO_2 in water for photosynthesis and growth of aquatic plants. The ability to take up nutrients via the roots is still very important for submerged aquarium plants, as water in an aquarium is often scarce in nutrients. Although the root system plays a major role in nutrient absorption, submerged foliage also can absorb considerable amounts of nutrient from water.

Another important character of water plants is that they can perform photosynthesis under low light intensity (700 to 1000 lux).

Aquatic ornamentals

Aquatic ornamentals are used for pond decorations and aquarium decorations. The choice of plant species purely depends on the purpose. The obvious reason for introducing different types of water plants to an aquarium is aesthetic. Arrangements made with different colour foliages give an attractive appearance. But a second reason is that aquatic plants produce considerable amounts of oxygen during photosynthesis, and thus help to improve the levels of dissolved oxygen in aquarium water. Leading oxygen producers in an aquarium are *Cabomba spp., Myriophyllum spp., Elodea spp.* and *Vesicularia spp.* A third reason is that the foliage of some aquatic plants, especially the *Cryptocoryne* species, acts as surfaces to which suspended particles in water adhere. These types of aquatic plants help to clean the water in the aquarium.

In aquascaping, many different aquarium plants of suitable varieties are arranged in a harmonious way to create an aesthetic finish. More popular ornamental plants in a modern day aquarium are:

- A*nubias;*
- *Cryptocoryne;*
- *Echinodorous;*
- *Aponogeton;*
- *Hygrophyla;*
- *Bacopa;*
- *Hydrilla;*
- *Cabomba;*
- *Myriophyllum;*
- *Lagenandra;*
- *Vallisneria;*
- *Nympheae; and*
- *Elodea.*

Among these species, *Cryptocoryne, Lagenandra and Aponogeton* are the most important aquarium plants of Sri Lanka.

Cryptocoryne

Aquatic plants can be divided into two groups: obligate submerged and amphibious plants. The obligate submerged plants cannot grow and thrive outside the aquatic environment. They collapse and dry out very fast when removed from the water.

The genus *Cryptocoryne* is an amphibious member of *Araceae* with more than 50 different species distributed throughout Southeast Asia. *Cryptocoryne* species are found mostly in slow moving fresh waters of streams and shallow rivers, of width 1-20 metres, in tropical forests. *Cryptocoryne* species provide unique functions for aquatic ecosystems, such as cleaning of water by adhering suspended particles, and prevention of soil erosion with their deeply growing rhizomes. Underground rhizomes can survive in severe drought conditions. These species are very popular as aquarium plants because they are quite easy to grow in aquaria and thrive well for a longer period. Moreover, they provide a wide range of colours (green to copper brown) and foliage patterns for a colourful and fascinating aquarium. No other aquarium plant can add reddish, reddish-brown or reddish-brown marble colours to an aquarium. Some of the stunted types of *Cryptocoryne,* such as *C. parva,* are used to create "lawns" in aquascaping. Thus *Cryptocorynes* are an intrinsic component of any colourful aquarium, and have an extended life span. This is the reason why more than 20 out of 53 identified species of *Cryptocoryne* are used for aquarium decorations in the world (Table 1).

Global distribution of *Cryptocoryne*

The *Cryptocoryne* species is mostly confined to the Asian region. *Cryptocoryne* species found in different countries and identified species for ornamental purposes are given in Table 1.

***Cryptocoryne* in Sri Lanka**

Ten *Cryptocoryne* species are found in and along springs, streams and rivers in the lowlands and midland rain forests of Sri Lanka and all are endemic to their respective regions. These species are not found anywhere else in the world as naturally occurring water plants. The natural habitats of these species are both submerged in water and emerged. They provide shade and shelter for fish and other aquatic fauna. Most of the endemic, threatened fish live associated with these endemic plants (Table 2).

The natural habitats of these species shrink dramatically as a result of uncontrolled and forbidden exploitation and rapid depletion of forest covers. For example, the island's forest cover, which was as high as 80 per cent in 1920, re

duced to 20 per cent by 1990. Even this remaining natural forest source is deteriorating day by day. As a consequence, nine *Cryptocoryne* species are under threat of extinction (Table 3).

Six species, namely *C. becketii, C. wendtii, C. nevillii, C. thwaitesii, C. willisii* and *C. undulata,* are very popular ornamental aquatic plants, with high export potential. The existence of solitary colonies of these species mostly confined to upper parts of rivers and streams and seldom downstream indicates that they are hardly dispersed via seeds.

Genus - *Lagenandra*

This green-coloured aquarium plant, also an Araceae member, is very popular among aquarium enthusiasts. The *Lagenandra* is an amphibious plant which grows in marshlands in the wet lowland and midland areas of Sri Lanka. Seven different species of *Lagenandra* have been identified in Sri Lanka, six of which are considered endemic (Table 4).

Unlike *cryptocoryne*, all *Lagenandra* are not popular or appropriate as aquarium plants. At present, only two species such as *L. thwaitesii* and *L. ovata* are exported from Sri Lanka. The species *L. thwaitesii* is very rare and reported to be found in "Sinharaja", a wet lowland virgin rain forest reserve. The existence of a silver line at the periphery of the leaves is the unique feature of this species. The other species of *Lagenandra* too are rapidly gaining popularity in the export market. Although streams and rivers in lowlands are also habitats of *Lagenandra,* the latter are seldom found in association with *cryptocoryne* in the same site.

Aponogeton in Sri Lanka

Aponogeton is a genus with more than 40 species distributed throughout the tropics, including Africa, Madagascar, India, Sri Lanka, Malaysia and Australia. These species are obligate submerged hydrophytes, with widely varying foliage that immediately collapses outside the water. The beautiful foliage patterns of *aponogetons* raise the ornamental value of an aquarium. Four *Aponogeton* species, namely *A. natans, A. jacobsenii, A. rigidifolius and A. cris-*

Table 1: Global distribution of *Cryptocoryne* and species used in aquariums

Country/ Region	Number of species	Species found
Sri Lanka	10	*C. beckettii * , C. wendtii*, C. parva * , C. willisii *, C. thwaitesii *, C. alba, C. bogneri, C. walkeri*, C. nevillii * , C. undulata **
India	05	*C. ciliata*, C. cognata*, C. consobrina, C. retrospiralis*, C. spiralis *.*
Malaysia	12	*C. affinis* , C. ciliata *, C. cordata *, C. decus-lvae, C. diderici, C. ellipfica, C. longicauda, C. griffithii, C. minima *, C. nurii *, C. purpurea, C. schulzei.*
Sumatra	10	*C. ciliata*, C. diderici, C. jacobsenii*, C. longicauda, C. minima , C. moehlmannii, C. pontederiifolia*, C. scurrilis, C. villosa, C. zukalii.*
Thailand	06	*C. albida , C. annamica, C. ciliata, C. crispatula, C. cruddasiana, C. vietnamensis*
Borneo	15	*C. auriculata, C. bullosa, C. ciliata , C. cordata*, C. edithiae, C. ferruginea, C. fusca, C. grabowskii, C. hudoroi, C. keei, C. lingua, C. longicauda, C. pallidinervia, C. striolata, C. zonata.*
New Guinea	03	*C. ciliata*, C. dewitii, C. versteegii.*
The Philippines	05	*C. aponogetifolia *, C. ciliata *, C. coronata, C. pygmaea, C. usteriana.*

** Species currently used in aquarium*

Table 2: Endemic fish species that live in association with aquatic plants

Scientific name	Common name	Sinhala name
Puntius bandula	Bandula barb	Bandula pothaya
Puntius nigrofaciatus	Black ruby barb	Bulath hapaya
Puntius asoka	Asoka barb	Asoka pethiya
Rasbora vaterifloris	Golden rasbora	Halmal dandiya
Belongtia signata	Combtail	Thalkossa
Malpulutta kretseri	Ornate paradise fish	Malpulutta

Table 3: Distribution and threatened status of *Cryptocoryne* species in Sri Lanka

Species	Locations	Threat status
*C. beckettii #**	Kandy, Kegall, Matale, Ratnapura, Gall, Hambantota	Highly threatened
*C. wendtii #**	Matale, Kandy, Kurunegala	Threatened
*C. Nevillii #**	Batticaloa, Ampara	Highly threatened
*C. undulata #**	Kandy, Kegall, Matale	Highly threatened
*C. parva #**	Kandy, Kegall, Matale	Highly threatened
*C. walkeri #**	Kandy - Peradeniya	Highly threatened
*C. x willisii #**	Kandy, Matale, Ratnapura	Highly threatened
*C. thwaitesii #**	Gall, Kaluthara, Ratnapura	Highly threatened
C. alba #	Gall, Ratnapura, Kalutara	Highly threatened
C. bogneri #	Ratnapura	Highly threatened and nearing extinct

** Species currently used in aquarium ; # Endemic species*

Table 4: Distribution and threatened status of *Lagenandra* species in Sri Lanka

Species	Locations	Threat status
L. ovata *	Gall, Kaluthara	Not threatened
L. praetermissa #	Kandy, Ratnapura	Highly threatened
L. lancifolia #	Kandy Ratnapura	Highly threatened
L. koenigii #	Colombo, Kaluthara, Gall	Highly threatened
L. jacobsenii #	Kegall, Gall	Highly threatened
L. thwaitesii # *	Kaluthara, Ratnapura	Highly threatened
L. bogneri #	Ratnapura	Threatened

** Species currently used in aquarium;.# Endemic species*

tocoryne and *Lagenandra*, *Aponogeton* thrive well in stagnant, shallow or deep water bodies like ponds, tanks, channels and temporary water sources like marshes, where they dominate the submerged vegetation. *Aponogeton* flower rather frequently throughout the year and give fruits with or without pollination by water. Even though they propagate invasively via seeds, collection of *Aponogeton* bulbs for the export market is rather widespread, leading to destruction of natural habitats. As a consequence, three species - *A. jacobsenii, A. natans and A. rigidifolius* - already have been identified as threatened. Yet they continue to be illegally removed and exported in large quantities.

History of the industry

The cultivation of ornamental aquatic plants has an obscure and undatable origin. It may have arisen as an incidental feature of the very ancient arts of pisciculture and landscape horticulture, which can be traced back to at least 2500 B.C. in Egypt, Assyria and Persia. Clearer emphasis was placed on the aesthetic value of water plants with the rise of Buddhism, when formal lotus pools became an integral feature of the gardens of all Buddhist temples. In Eastern Asia, water gardening reached a peak of excellence through the supreme artistry and symbolic intricacy of the designs of Chinese and especially Japanese landscape gardeners. It was much later that the art of water gardening spread throughout the western world. Popular enthusiasm for water gardening in Britain has grown since 1900 and since World War I there has also been marked enthusiasm in the USA, South-East Asia and Russia for the cultivation of ornamental plants for aquarium purposes.

The industry in Sri Lanka

Many important plant species that are used for aquarium decorations in European countries are found only in the natural habitats of Sri Lanka. That is the foremost reason for the high export demand for Sri Lankan aquatic plants. Six companies are engaged in the aquatic plant industry, but unfortunately only a few of these cultivate local plant species and export them as potted plants. The majority of the aquatic plants are collected from the wild and exported in bulk. The species include *aponogeton, nymphaeae, cryptocoryne* and *lagenandra*. While it is not possible to determine the precise quantity of exports of each plant species, a total of about 8 million of aquatic plants were exported in 1998. And, while there is enough potential for cultivation of aquatic plants required by industry, wild collection continues.

Collection of aquatic plants

Many aquatic plant export industries have regular collectors and suppliers. The collectors know the natural habitats for specific plant species in forest reserves and rural areas. In addition, organized groups resort to large-scale exploitation. Many are not aware of the importance of these plants and they collect them to meet daily living expenses. They tend to remove these plants regardless of the environmental damage, destroying the natural balance of the ecosystem. Many plant species, including *Cryptocoryne, Aponogeton* and *Lagenandra* that are endemic but not listed as threatened species in 1998, become highly threatened in the year 1999 because of the ruthless exploitation (IUCN list of threatened fauna and flora of Sri Lanka, 1999).

Impacts on natural ecosystems

Fresh water ecosystems of tropical rain forests are rich in biodiversity. They have many flowering plant species and water ferns as flora, while fresh water fish, amphibians, crustaceans and reptiles are the important fauna. Many indigenous fish species are found in these natural habitats (Table 4). The removal of even a single aquatic plant species from these communities can unbalance the ecosystem and cause valuable genetic resources to disappear. The most unfortunate part is that many species of *Aponogeton, Cryptocoryne* and *Lagenandra* are collected along with the soil and as a consequence other fauna and flora are also affected. From a long time ago these plants have formed a live mat over riverbanks and streams, preventing soil erosion even under heavy rain conditions. Once these natural covers are removed, banks of rivers and streams are subjected to heavy erosion, and that, in turn, leads to many other environmental hazards like floods and silting of water reservoirs. The most vulnerable genus in Sri Lanka, at present, is *Cryptocoryne*, all species of which are endemic and nine highly threatened. Nevertheless, almost all the species are still collected from the wild and exported regularly for aquarium decorations.

Impacts on aquarium industry

The Sri Lankan aquatic plants industry exclusively depends on endemic species of *Cryptocoryne, Lagenandra* and *Aponogeton*. It is questionable whether industry can survive or improve through wild collection, which leads to depletion of plants in their natural habitats. The government has therefore imposed stringent rules and regulation to protect the natural habitats. Of vital importance now is the development of methods for propagation and cultivation of plants suitable for industry while protecting the natural environment.

Tissue culture of aquatic plants

Plant tissue culture has made a significant contribution to plant biotechnology. Its use has lead to several practical gains to agriculture and industry. Tissue culture has been successfully employed

for micro-propagation of a wide range of plants, including cut flowers, foliage plants and many horticultural plants.

While appreciable progress has been made with land plants, however, the results for aquatic plants have not been impressive. Published reports on the tissue culture of water plants are limited. The methodology was developed by our institute to mass propagate five endemic aquatic plant species (*C. beckeetii, C. wendtii, C. nevillii, C. thwaitesii and C. willisii*) through tissue culture technology.

Most water plants do not produce seeds, and plants produced by natural vegetative propagation are not adequate to meet the demands of industry. Tissue culture is a suitable method to mass propagate plants according to a scheduled production programme.

Introduction of tissue culture to produce these valuable aquatic plants holds several advantages for industry. It will provide good quality plants without pest or disease at a competitive price for the export market, while conserving aquatic plants in their natural habitats. Large-scale plant production can be programmed according to export requirements and preservation of plant species *in vitro* is also possible.

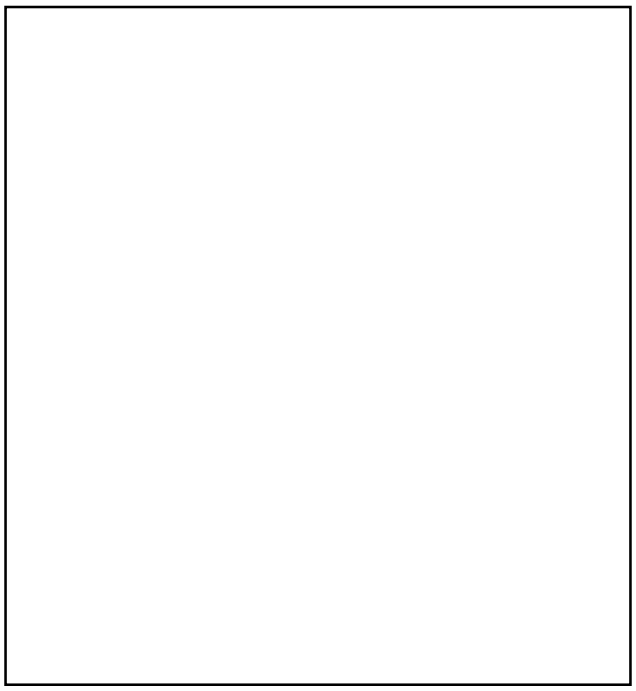
Stage 1: Culture establishment

Cryptocoryne rhizomes are collected and washed with household detergent prior to surface sterilization with chemical sterilant. These sterilized rhizomes are successfully grown with 50 per cent recovery in culture tubes containing nutrient media with plant hormones. In the case of *Lagenandra thwaitesii*, germinated seedlings with four leaves are used as explants.

Stage 2: Multiplication of shoots

Nutrient media with different plant hormones at various concentration levels were tested to improve shoot multiplication of *Cryptocoryne* and *Lagenandra* cultures. The modified MS medium gave the highest shoot multiplication (5.8 and 4.2 shoots respectively) of *Cryptocoryne* and *Lagenandr*a. Further optimization of plant hormone level in multiplication medium gave the highest shoot multiplication of *Cryptocoryne* (60 shoots) within 10 weeks (Figure 1). The shoot multiplication of *Cryptocoryne* was consistently increased with subculture.

Figure 1: Shoot multiplication in liquid medium



Stage 3: Rooting

Shoots of tested aquatic plant species multiplied *in vitro* are easily rooted in a nutrient medium containing activated charcoal. All species show 100 per cent rooting within 4 weeks, with good plant vigour.

Stage 4: Acclimatization and field planting

Hardening or acclimatization of tissue-cultured plants is the most important aspect that determines commercial feasibility in terrestrial plants. Even though aquatic plants grow in an aqueous environment they require good care at this stage. Generally *Cryptocoryne* and *Lagenandra a*re grown in deep water tanks before being packed for export markets. But this method has many disadvantages. For example, uncontrollable algae formation, difficulty in pest control and application of fertilizer are major disadvantages. Water requirements and wastage are very high. We have therefore introduced an alternative method for acclimatization and maintenance of plants until they are exported. Water up to about 2 or 3 inches at the bottom of the tank is used to keep the potted plants, which are placed in a wire mesh. This system is completely covered with polythene. We found a 100 per cent plant survival in the polythene propagator system, whereas in submerged treatments it was about 90 per cent. The polythene propagator system can therefore be recommended for amphibious types aquatic plants.

The advantages of using the polythene propagator system are as follows:

- Maximum plant survival during acclimatization.
- Lower quantity requirement of water than in deep-water system.
- Avoidance of water pollution. What happens with current methods is that a large quantity of water gets polluted as it mixes with fertilizer and agrochemicals. This pollutes the natural aquatic environment highly in several ways.
- Avoidance of algae problem. Formation of algae is an inherent problem when cultivating aquatic plants in tanks full of water. But in unsubmerged polythene-covered propagator, water is available only under the foliage, therefore the algae problem does not occur.
- Fertilizer application, pest and disease control, and other cultivation practices are easier due to their direct access to plants.

A protocol has been developed that is suitable for commercial level production

of *Cryptocoryne* and *Lagenandra* species using tissue culture (Figure 2). Mass production of aquatic ornamental plants using tissue culture will be beneficial for both the aquatic plants industry and the environment.

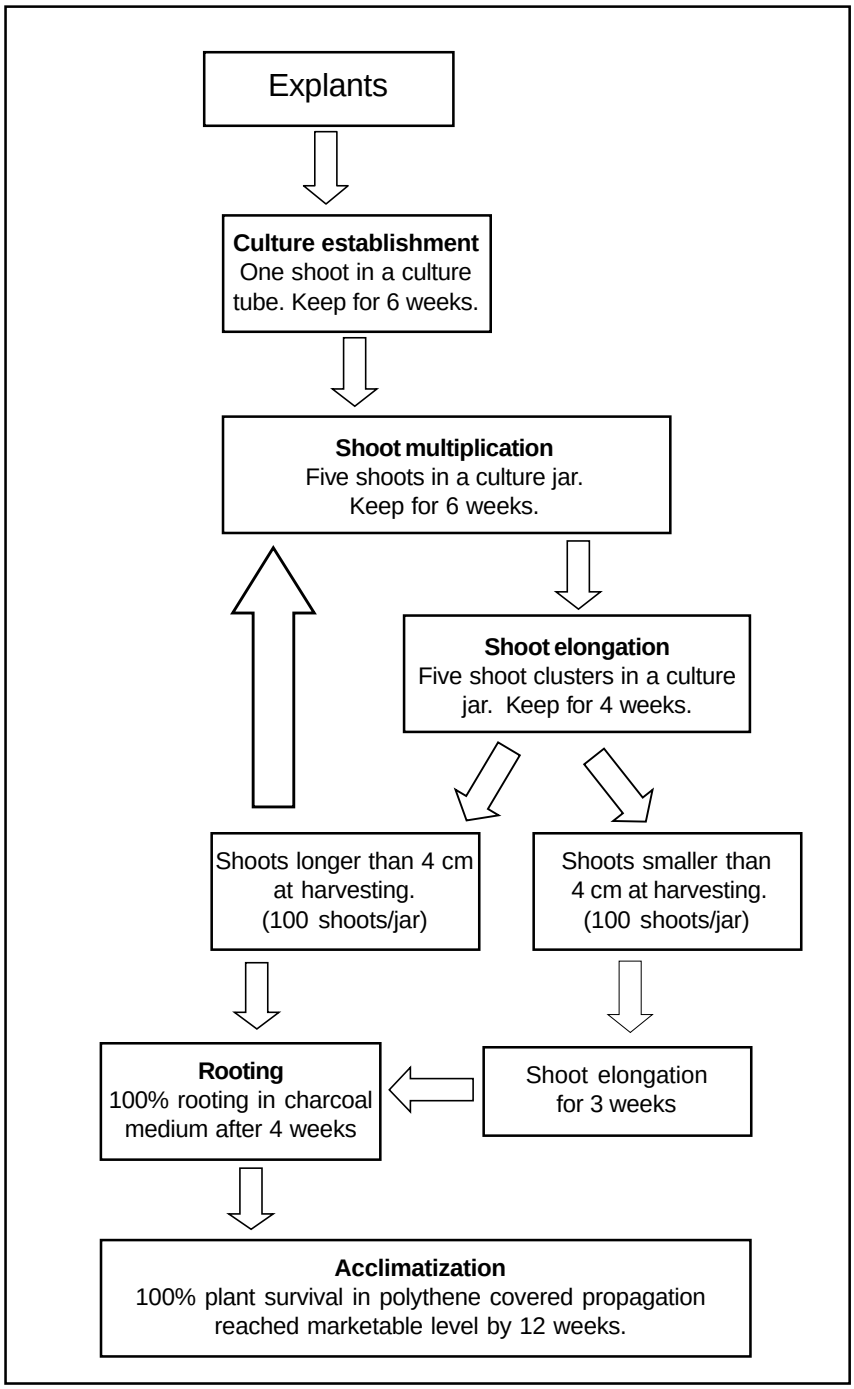
Benefits to industry

- High quality aquatic plants can be produced at a cost of Rs. 5 - 7 for the export market.
- Quarantine procedure during exports is easier as tissue-cultured plants do not contain soil particles, pests or disease-causing organisms.
- It is possible to produce the large number of plants required by the export industry.
- The production cycle can be programmed regardless of seasons and adverse weather conditions.

Benefits to environment

- Production of tissue cultured plant species for the export market will reduce the pressure on their natural habitats.
- It will facilitate the conservation of rare and threatened species like *Cryptocoryne* and *Lagenandra.*
- Mass production of plants is possible with just a few mother plants at the initial stage. By repeating the procedure a sufficient number of plants can be raised.
- A replanting scheme can be initiated to rehabilitate the natural locations.

Figure 2: Production flow chart



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