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Liquid Culture System: An Efficient Approach for Sustainable Micropropagation

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Abstract

Micropropagation of important forestry, horticultural and medicinal plants have made revolutionary changes in terms of research and commercialization. However, there are a variety of factors which influence the scaling-up and commercialization aspects, which decide whether mass propagation will be effective and lucrative. Low rates of shoot multiplication, increased costs of media components, loss of cultures due to contamination, and difficulties with hardening and acclimation are the key obstacles to scaling up micropropagation technology. These restrictions have forced a large number of *in vitro* technologies developed for a range of plant species to be used only under research laboratories settings. To apply tissue culture technology to large-scale propagation, it is required to develop techniques that are relatively simple to adopt, have high multiplication rate with high levels of reproducibility, and exhibit higher survival of plantlets when transferred to *ex vitro* conditions. Efficient techniques include utilization of liquid culture systems and replacement of agar with other gelling agents. These techniques allow development of micropropagules that not only function better in *post-vitro* soil conditions and are comparatively less expensive, but will also help develop a workable micropropagation technique that can be applied to the mass production of desirable plant species. The current review describes liquid culture system as an efficient approach to produce large number of plants at low production cost.

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Introduction

In the last decade, plant tissue culture has proved its significance in several areas of research and commercialization. These include: a) CRISPR-CAS-9 mediated improvement of crops, b) commercial production of horticulture and medicinal plants, c) transgenic plant development, c) *in vitro* production of important secondary metabolites, d) production of novel varieties through embryo rescue and haploid culture, e) germplasm conservation.[1] Tissue culture methods are generally used to improvise the plants which do not produce seeds or have stubborn

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seeds that can't be stored in seed gene banks under normal capacity settings. In order to maintain quality of plantlets, *in vitro* procedures have been proved to be very useful in various roots and tubers, ornamental plants, medicinal plants, and several other tropical fruit plants. Plant tissue culture was initially utilized as a research method with a primary goal of cultivating and studying the development of tiny, isolated plant tissue pieces or isolated cells.[1] Plant tissue culture has gone through numerous stages of progress, including logical curiosity, a research tool and innovative applications, similar to other advanced methods. Micropropagation technology is a technique for *in vitro* propagation of plants by using principles of biotechnology. The plants are derived from taking initiation material like stem part, root part or leaf tissues and the technique developed guides in large scale production of economically important crops varieties. Singh *et al.* (2016) has enlisted key characteristics of micropropagation technique, which include a regulated environment, managed plant growth, and product (micro-propagules) that are free of many pests and diseases.[2] Due to the propagated plants' compact size, nursery space and plant transportation expenses are reduced. The biggest drawback of tissue culture plants is how expensive they are to produce. A variety of plants intended for commercial tissue culture propagation are constrained by this challenge.

Plant organs and tissues are cultured *in vitro* on artificial media, which supply the nutrients vital for development. The progress of micropropagation as a method for plant propagation is enormously impacted by the type and concentration of the culture medium components used. Generally, in any tissue culture medium, the components are majorly classified in four groups excluding sucrose (carbon source) and agar (solidifying agent). These groups are: a) Macronutrients (Nitrogen, Potassium, Phosphorus, Magnesium, Sulphur), b) Micronutrients (Manganese, Boron, Zinc, Cobalt, Copper), c) Iron and chelating agents ($FeSO_4$ and EDTA) and d) organic supplements (vitamins and amino acids). The most commonly utilized medium is the one described by Murashige and Skoog (1962). This medium was ideally developed for growth of tobacco callus and later on it was proved beneficial for wide range of species with slight modifications.[3]

In addition to these inorganic supplements, plant tissue culture medium often provides a carbohydrate (sucrose is typically standard) to substitute the carbon that a plant normally fixes from the air through photosynthesis. As mentioned above, numerous media also include various organic substances, vitamins, and plant growth regulators to promote development. In early trials of research in development of growth media, undefined components like natural plant products, yeast extracts, protein hydrolysates *etc*. were utilized instead of defined nutrients or amino acids, or even as additional supplements. Coconut milk, for example, is still frequently utilized, and banana homogenate has been a famous expansion to media for orchid culture. There are certain precautions, which are needed to be followed while designing and development of medium. For example, there should not be any changes in laboratory conditions and type of inorganic or organic salts (for instance the hydration of compounds). Table 1 shows general composition of plant tissue culture medium, which is followed by most of the researchers.

Table 1: List of inorganic/organic salts, carbon source, vitamins, and solidifying agents used in generalized plant tissue culture media

Sr. No.	Components	Elements	Inorganic/Organic salts
1	Macro-elements	Nitrogen (N)	NH_4NO_3
			$(NH_4)_2SO_4$
		Potassium (K)	KNO_3
			KCl
			KH_2PO_4
			K_2SO_4
		Calcium (Ca)	$Ca(NO_3)_{2.}4H_2O$

			$CaCl_2.2H_2O$
		Magnesium (Mg)	$MgSO_4.7H_2O$
		Sodium (Na)	Na_2- EDTA
			$NaH_2PO_4.2H_2O$
			Na_2SO_4
		Ferrous (Fe)	$FeSO_4.7H_2O$
2	Micro-elements	Potassium (K)	KI
		Boron (B)	H_3BO_3
		Ferrous (Fe)	$Fe_2(SO_4)_3$
		Manganese (Mn)	$MnSO_4.4H_2O$
		Zinc (Zn)	$ZnSO_4.7H_2O$
		Sodium (Na)	$Na_2MoO_4.2H_2O$
		Copper (Cu)	$CuSO_4.5H_2O$
		Cobalt (Co)	$CoCl_2.6H_2O$
3	Vitamins	Calcium pantothenate	
		Thiamine HCl	
		Inositol	
		Nicotinic acid	
		Pyridoxine HCl	
4	Amino acids	Glycine	
		Cysteine HCl	
		Glutamine	
5	Carbon Source	Sucrose	
6	Solidifying agents	Agar	

Liquid Medium as an Efficient Approach for Tissue Culture

In plant tissue culture, generally semi-solid medium is used for regeneration and other purposes. However, there are several constrains in using semi-solid medium for example, high production cost, less protocol efficiency and multiplication rate, high contamination rate and somaclonal variations, which arise during culture conditions.[4] The high prices of media have restricted the widespread adoption of the plant tissue culture application.[5] Low plantlet production rates, high labour costs, and increased space requirements continue to be the barriers in adoption of semisolid media for commercial production.[6] In order to produce valuable and affordable *in vitro* plantlets, the proper selection of media components should be taken into account. Liquid culture media have been employed as an effective way to address the problems which arise during the use of semisolid medium and also enable the researchers or commercial producers in development of automation and cut down both time and cost.[5,7] Uniform culture conditions, quick media replacement without changing the container, sterilisation with ultra-filtration, and simpler container cleaning after use are all benefits of liquid culture systems. Agar culture media require surface culturing of tissues, whereas liquid culture media allow for the use of containers of various capacities.[8,9] Faster growth rates, efficient nutrient absorption by tissues, and dilution of secreted growth inhibitors, such as phenolics produced by explants, all represent potential advantages of liquid culture systems over solid cultures.[10]

Plant tissues/ explants of various species have shown improved performance in liquid medium as opposed to solid or semi-solid medium.[11] *Acacia nilotica* shoot numbers were around ten times higher in liquid culture than in gelled culture.[12] However, liquid culture is characterised by excessive humidity and a restricted exchange of gases between the interior atmosphere of the culture vessel and its surroundings. These circumstances might lead to physiological illnesses such as hyperhydricity. If liquid culture is defined as the growing of explants on a nonsolid media, then other changes to the fundamental system are feasible. Explants are placed in a static liquid solution in the most basic liquid culture methods. For instance, thousands

of embryos may be produced from androgenic pollen grains of wheat in a static liquid culture. Modifications include aeration, which involves bubbling air through the medium, use of a support for the explants (such as cellulose substrate), shaking the culture to maximize contact between the medium and explants. Temporary immersion system, in which the explants are submerged and removed in the medium for varying lengths of time are also very efficient methods apart from use of bioreactors (closed or open). Scaling up and utilization of bioreactors for commercial production, as well as the development of organ-genic propagules like bulblets, have all been studied in detail.[13,14] While it is outside the purview of this work to evaluate all occurrences and implications described in liquid culture, the process by which liquid culture can control plant growth and development is covered. Growth rates and morphogenetic patterns are used to demonstrate the advantages of liquid culture over traditional gelled medium, while the drawbacks are underlined. The role of certain chemical variables in coordinating growth and development is also discussed.

Explants cultivated in solid media will display polarity in their response and cells that are not in direct touch with the medium must absorb nutrients and process regulatory signals by diffusion from nearby cells. This may result in the peripherally placed cells sensing a different signal. Since the entire surface of the explant is immersed in the medium and may thus sense chemical signals, this condition is avoided in liquid culture. The decrease in the harmful effects of toxins is another benefit of adopting liquid systems for culturing. Any metabolite that the explant releases into the media may have harmful or inhibiting effects on subsequent growth and/or development. Toxins quickly dilute in liquid systems, as opposed to solidified media, where released compounds stay near to the explant, lessening any possible inhibitory impact. Liquid cultures often have higher rates of multiplication and proliferation than traditional gelled cultures. According to Kim *et al* (2003), garlic shoots on solidified cultures showed a decrease in the growth rate and fresh weight of shoots.[15] Explants produced on liquid medium for potato micropropagation showed faster shoot development rates than explants cultured on solidified media.[16] Sugarcane meristems were cultivated in both liquid and solid medium as well as in a transient immersion system, and[17] compared the growth rates of these meristems.[15] In some cases, strangely, the growth rates of the liquid and solid media did not differ much, whereas the temporary immersion system yielded a growth rate that was double than that of the other two techniques.[18]

In liquid culture, shoots of *Pinus radiata*, tea, wild pear, and *Calotropis gigantea* have all shown better rates of multiplication, when compared with explants grown on agar-solidified media.[19-21] When cultivated in a liquid-shake culture, aspen root cultures quickly increased in biomass.[22] But faster development rate in a liquid environment is not a general occurrence. Suspension culture offers a lot of promise for reproduction and the generation of synthetic seeds since it can result in the formation of many somatic embryos. After just eight weeks in culture, one gram of embryogenic callus from coffee leaves may generate 1.2×10^5 somatic embryos under ideal development circumstances. According to Gawel *et al* (1990), liquid cultures generate more cotton somatic embryos than gelled cultures do.[23] A number of factors have been put forth as the causes of this improved growth rate, including better nutrient availability,[16,24] increased water availability,[25] a less pronounced gradients in nutrients and endogenous hormones, and a more gradual pH shift throughout culture,[23] removal of polarity, and a lessened impact of toxins. According to Singha (1982), decreased diffusion resistance and tighter contact between the explant and culture media lead to greater availability of nutrients and water.[24] However, there was no appreciable difference in the water content of micropropagated potato shoots between liquid and solid cultures. Increased carbohydrate and organic nitrogen build up led to an increase in shoot fresh weight, indicating that liquid culture favours nutrient digestion.[16] Depending on the species, kind of explant, and particular culture circumstances, growth rate increases may be caused by increased carbohydrate build up, increased water intake, or a combination of the two.

In contrast to gelled cultures, where depletion zones (gradients) do form around the explant, agitation of a liquid culture enables uniform dispersion of nutrients and growth-promoting agents. This is helpful because in agitated liquid cultures, the concentrations can be maintained uniformly, whereas the action of exogenously administered growth regulators frequently fluctuates with

concentration. Additionally, culture agitation results in higher explant aeration and, thus, increased growth rates26. Microspores are grown in a liquid media with developing ovaries to produce haploid wheat plants. As a "nurse culture," the ovaries release substances needed by growing microspores to finish androgenic development and form a haploid embryo.[27] Nevertheless, an extract made from immature ovaries did not promote androgenesis, indicating that ovaries actively create the necessary factor(s) in response to the physical environment of the liquid media. In order to ensure that all elements and regulators are dispersed uniformly throughout the media, androgenic differentiation will profit from the diminished barrier to diffusion.

Regeneration in Liquid Culture System

Somatic embryogenesis is the process through which a non-zygotic cell grows into a bipolar structure that resembles a zygotic embryo without having a vascular link to the original tissue.[28] In order to research different facets of embryogenesis, somatic embryos are employed as a model system. The possibility for extensive vegetative reproduction is perhaps the biggest benefit of creating somatic embryos. This method enables the production of genetically homogeneous plants from a superior parent as well as the multiplication of plants that are thought to be challenging to replicate. Furthermore, research involving genetic transformation benefit from the generation of a lot of embryogenic calli in liquid culture.[29] Establishing the proper kind of suspension culture is necessary for somatic embryogenesis to take place in liquid culture. Large vacuolated cells in suspension are frequently produced by undifferentiated callus and perish after two weeks. When the callus is triggered on a media that contains an auxin like 2,4-Dichlorophenoxyacetic acid (2,4-D) however, an embryogenic suspension can be produced; these cells are typically smaller and have dense cytoplasm. The development of embryogenic callus occurs in asparagus when the kind and ratio of the hormones alter. When the hormone combination of Indole Acetic acid (IAA), Benzyl Adinine (BA), and 6-(γ,γ-Dimethylallylamino)purine(2-iP) was substituted with kinetin and 2,4-D, globular callus clumps were seen. In contrast, *Ophiopogon japonicus* suspension cultures do not need plant growth regulators to produce somatic embryos.[30]

For the formation of somatic embryos and subsequent plant regeneration in some species, huge numbers of embryogenic cells or cell clumps are produced in suspension cultures, filtered, and then plated on solid media.[31] In these situations, the liquid phase of the culture serves just to promote cell multiplication, while keeping the individual cells and cell clumps in an embryogenic condition. In their study, Jayashankar *et al.* (2003), examined somatic embryos produced in both solid and liquid environments. These authors noted that although embryos derived from a solid media had big cotyledons, a poorly formed suspensor, and a relatively underdeveloped concave apical meristem, those obtained from a liquid medium had smaller cotyledons, a distinct suspensor, and a flat-to-convex shoot apical meristem. Embryos from the liquid media did not demonstrate dormancy, in contrast to those from the solidified medium, and they had high rates of plant regeneration.[32] What characteristic of the liquid medium is able to keep cell clumping in an embryogenic state is still a mystery? It is most likely a result of several variables working together. For instance, increased availability of metabolites and growth-regulating compounds that can be absorbed by all areas of the explant (owing to closer contact with the medium) along with lowered nutritional gradients all likely help to preserving the embryogenic potential of the culture.

Secondary Metabolites Production in Liquid Culture System

Plants create a wide range of organic substances known as secondary metabolites to help them interact with their biotic environment and develop defensive mechanisms.[33,34] The majority of secondary metabolites, including terpenes, phenolics, and alkaloids, are categorised based on their biosynthetic origin, exhibit a variety of biological activities, and are employed as biopesticides, agrochemicals, medicines, flavouring agents, perfumes, colours, and food additives. Field cultivation for the purpose of producing secondary metabolites has a number of drawbacks, such as poor yields and concentration swings resulting from environmental, seasonal, and geographic differences. In order to produce secondary metabolites, plant cells and cultures have therefore become appealing alternatives (Table 2).

Table 2: Reported plants species producing secondary metabolites under in vitro conditions

Sr. No.	Plant Species	Secondary metabolites reported	References
1.	*Capsicum chinense*	Capsaicin	[35]
2.	*Salvia castanea*	Tanshinone	[36]
3.	*Papavar orientale*	Morphine	[37]
4.	*Astragalus membranaceus*	Isoflvonoid	[38]
5.	*Psoralea corylifolia*	Daidzin	[39]
6.	*Bacopa monnieri*	Bacoside	[40]
7.	*Catharanthus roseus*	Vinblastine, vincristine	[41]
8.	*Chlorophytum borivilianum*	Saponin	[42]
9.	*Camptotheca acuminata*	Camptothecin	[43]
10.	*Isoplexis canariensis*	Canarigenin, uzarigenin, digitoxigenin, xysmalogenin	[44]
11.	*Ruta graveolens*	Psoralen, bergapten, xanthotoxin, isopimpinellin, imperatorin, umbelliferon	[45]
12.	*Salvia officinalis*	Carnosol, carnosic acid, rosmarinic acid	[46]
13.	*Tripterygium wilfordii*	Triptolide, wilforgine, wilforine	[47]
14.	*Rosa hybrida*	Anthocyanin	[48]
15.	*Panax ginseng*	Ginsenoside	[49]
16.	*Genista tinctoria*	Isoflavones	[50]
17.	*Nothapodytes nimmoniana*	Camptothecin	[51]
18.	*Ruta graveolens*	Psoralen, bergapten, xanthotoxin, isopimpinellin	[52]
19.	*Securinega suffruticosa*	Securinine, allosecurinine	[53]
20.	*Withania somnifera*	Withanolides	[54]

Bioreactors

Plant tissue, cell, and organ cultures have been acknowledged as potent tools for the clonal propagation of commercially significant crops (micropropagation), the production of valuable secondary metabolites, the expression of complex foreign proteins (molecular farming), and phytoremediation of waste waters (Phyto transformation and phytoextraction). It is possible to cultivate plant cultures on a large scale using liquid media *in vitro* under controlled environmental conditions in bioreactor systems. These plant cultures can be differentiated (embryos, shoots, seedlings, transformed or adventitious roots), or dedifferentiated (suspended cells). The main goal of the strategy is to produce as much plant biomass as is economically viable, ready for immediate use or for later separation of valuable products.

The bioreactor is a piece of specialised technology that controls numerous physical and/or nutritional parameters to enable intense culture. Systems using bioreactors typically include a culture vessel and an automated control block. The culture vessel is made to hold the grown cells in an aseptic environment while enabling options for maintaining ideal micro-environmental conditions, nutrients, and gaseous mass transfers to ensure their maximum development. The automated control block is a computerised, fully automated or semi-automated system that is intended to monitor and regulate the cultivation conditions in the culture vessel, including the agitation speed, temperature, dissolved oxygen and carbon dioxide (CO_2) concentrations, illumination regime, pH, composition of the overlay gaseous environment, and the level of the liquid medium. Existing bioreactors may be divided into four major categories based on the makeup of the environment in which the grown cells are housed: liquid-phase bioreactors, gas-phase bioreactors, temporary immersion systems (TIS), and hybrid bioreactors. The cultured cells/ tissues are fully submerged in a liquid nutrient solution in liquid-phase bioreactors. The best researched systems

at the moment are liquid-phase bioreactors, which include mechanically agitated, pneumatically agitated, hydraulically agitated, and membrane bioreactors. These systems have nearly infinite potential for use in generating undifferentiated plant cell suspension cultures.[55]

However, liquid-phase bioreactor methods often are unable to guarantee adequate development of differentiated plant *in vitro* systems. Because of hypoxia and hyper-hydricity, total submersion of plant tissue or organ cultures in the liquid media frequently results in deformities and material loss. Asphyxia and hyper-hydria are unfavourable physiological states that are solely brought on by the culture media's low oxygen concentration and water potential.[56] The creation of bioreactors with a sophisticated design, capable of supplying a specialised microenvironment in order to ensure the growth and physiological integrity of the cultures, is necessitated by the complex morphology of differentiated plant tissue and organs.[57] Gas-phase bioreactors TIS, and hybrid bioreactors[58,59] have been created to solve the problems that currently exist. The goal of TIS is to decrease physiological problems and retain the morphological integrity of fast-growing differentiated plant *in vitro* cultures by creating an ideal environment, improving nutrition and gas exchanges, and lowering mechanical stress. In TIS, explants are regularly submerged in a liquid media and subsequently exposed to a gaseous atmosphere, providing the most natural environment for plant tissue and organ *in vitro* cultures[57] TIS has been developed in many forms and is often used in the commercial micropropagation of commercially significant plant species. TIS have also been used in the study of secondary metabolite synthesis, molecular farming, and even phytoremediation of hazardous substances[57] because to its straight forward design and adaptable functioning.

Temporary Immersion System (TIS): A Modification In Liquid Culture System

The original idea for TIS was developed by scientists in 1983, when they created a device called "auxophyton" that could combine aeration and liquid media cultivation.[60] Auxophyton rotated the culture containers on a wheel, alternating exposing the test plants to air or submerging them in liquid. The carrot tissue was 2.6 times heavier after 20 days than the tissue grown on an agar medium.[5] Earlier attempts in growing carrot tissue cultures completely immersed in water failed, probably for lack of oxygen.[61] TIS-based bioreactors have experienced several developments since that time. However, every device complies with the specifications given by Teisson *et al.,*[62] including: (a) no continuous immersion, (b) sufficient mixing and OTR, (c) consecutive medium changes and automation, (d) low shear stress, contamination and costs. Different plant species have indicated that TIS has good impacts on shoot proliferation,[63] shoot vigor,[64] SE,[65] plant material quality.[66] as well as micro cuttings and microtuberization.[67,68] The most important factors determining the effectiveness of TIS are hyperhydricity and adjusting the immersion time.[69,70]

Hyperhydricity: a Disadvantage in Liquid Culture System

Although liquid culture techniques often promote greater, more rapid multiplication and biomass build up, there are several species that are not suited for liquid cultures because they are more likely to exhibit physiological abnormalities called hyperhydric syndrome.[71] The normal soil environment is produced by solid medium for terrestrial plants. Usually, explants that are totally submerged in growing media have the morphological alterations common to plants from liquid environments. Hyperhydricity, a condition where plants store too much water in their tissues, can occur in these situations.[72] The stems of hyperhydric shoots are transparent and brittle, contain a lot of water, and have a severe lack of chlorophyll, among other physiological abnormalities. Shoots with excessive water content frequently have thick, elongated, curled, and wrinkled leaves.[73] They have fewer layers of palisade cells, wide intercellular gaps, chloroplast degeneration, uneven stomata, and a very thin cuticle with less cellulose when seen anatomically. Because there is so much water in the apoplastic gaps of hyperhydric tissues, they have reduced dry biomass.[74]

The plant material may occasionally still be unable to develop even after the transfer of the hyperhydrated tissues to the solid medium to restore the plant to a normal state.[75] Later phases of plant growth may experience ramifications from the vitreous effect. Additionally, proliferating hyperhydric shoots have trouble establishing roots, as seen in the cases

of *Salvia officinalis*[46] and *Centaurium erythraea*.[75] This is not always the case and can be observed as exception as we can see in other plants, like in case of *Catharanthus roseus* liquid culture system did not prevent the subsequent formation of roots on the shoots but actually aided in the process.[41] The shoots also rooted more quickly, showed higher percentages of rooted shoots and numbers of roots on a single shoot, and were longer than the plants grown in the solid medium. Most of the time, the process of acclimatising rooted shoots is independent of the consistency of the growing medium and has no impact on the survival rate. Even more has been said about how rooting in liquid may forecast a later stage of micropropagation since there is less chance of root system injury during soil transplanting.[41] A large decrease in the generation of bioactive metabolites in the morphologically altered organs may be another unfavourable effect of hyperhydricity.[76] This phenomenon manifests as a result of several stressful circumstances, such as extreme humidity. A gaseous restricted environment with low oxygen concentration may cause hyperhydricity. Hypoxia can result from the extra water in the tissues reaching low saturation levels.[19] Free radical-induced oxidative stress can harm tissues and cells and interfere with their metabolic processes. Normal development is disturbed as a result of all these variables. Agitation might produce aeration, however certain species are delicate to the shear stress and mechanical damage brought on by shaking the culture. Hyperhydricity may be markedly increased by exogenously applying cytokinin to a liquid media, especially at high doses.[77] In the meantime, the medium is frequently supplemented with cytokinin to promote the development and proliferation of *in vitro* shoots.

Role of Support Matrix in Liquid Culture System

Many methods for supporting plants over stationary liquid to lessen hyperhydricity have recently been investigated. Support matrix facilitates continuous and simple nutrient absorption, while permitting shoot development at very high levels of aeration. It enables dangerous phenolic exudates to spread throughout the media. Furthermore, the shear stress and mechanical damage brought on by the aeration and agitation associated with shake flask cultures are eliminated by the supports' static nature.[78] For most plant systems to multiply, root well, and anchor better in various types of culture containers, some sort of solid matrix is also fundamentally necessary. The addition of expensive gelling agents, as well as the cost of washing and cleaning, are avoided when support matrices are used. The likelihood of contamination can be decreased during the maintenance of these types of cultures since subculturing is only possible with the addition of sterile liquid medium.[79,80] However, when employed, a mechanical support should be porous, inert, non-toxic, resistant to plant digestion enzymes, and autoclavable. There are currently several mechanical supports available, and many people have successfully used them in various industrial systems. The majority of the time, a significant decrease in manufacturing costs favoured overall growth. For instance, cotton fibre costs around $2/kg, whereas agar costs between $100 and $200/kg. Similar to that, apple rootstock was rooted using a matrix made of sugarcane bagasse.[81] A high-quality plant cultivated on sugarcane bagasse was significantly (13.4%) less expensive than one grown on agar-gelled media. A cost reduction of roughly 35% was made possible when the quantity of high-quality rooted plants exceeded 1000. In order to cultivate ginger and turmeric at a lower cost than agar,[82] successfully employed glass beads. In his testing, there was a 94% decrease in the cost of the media. He also showed that only 15 to 18 ml of media were needed per culture container (an Erlenmeyer flask with a 100 ml capacity) when glass beads were employed as support matrices. As a result of this technique, the price of medium was significantly reduced because one litter of media created 50 cultured vessels (only 30 containers are filled in the case of agar-gelled semi-solid medium).

Plants of ginger and turmeric proliferated as well on liquid glass bead media as they did on agar-based medium. For vanilla, a similar kind of reaction was seen. Even with a modest vitrification, *Ficus cv.* "Mini lucii" had a greater rate of multiplication. On glass bead liquid-medium, *Saintpaulia, Syngonium, Philodendron*, and *Spathiphyllum* also showed faster multiplication rates and better growth.[82] Glass beads were employed by McLeod and Nowak (1990) to propagate raspberry and white clove plants, and they claimed a 60 percent media cost savings as a result. Glass beads were effective in maintaining callus and shoot organogenesis in *Rhododendron.* After being washed with acid, the beads can be utilised again.

An effective strategy for the speedy and inexpensive *in vitro* multiplication of certain commercially relevant plant species was glass bead-supported liquid media, such as *Celastrus paniculatus*, *Chlorophytum borivilianum, Terminalia bellerica*, and *Boswellia serrata.* In all of these plants, liquid medium encouraged shoot multiplication, shoot elongation, and accumulation of total fresh and dry weight. The shoots raised in this medium had a greater number of leaves, each with a bigger surface area and thicker laminae. For *C. paniculatus* and *B. serrata* shoot cultures, an increase in chlorophyll a, b, and total chlorophyll content was seen. The use of glass beads was very successful and did not result in any degradation due to hyperhydricity in liquid culture. Plantlets may be easily removed from the media due to use of glass beads. The support matrix used by different researchers has been listed in Table 3.

Table 3: Different mechanical support types are utilized at various phases of micropropagation of various plant species.

Sr. No.	Different supports systems	Micropropagation stages	Plants	References
1	Cotton fibre	Callus organogenesis	Artemisia annua	[83]
2	Filter paper bridges	Multiplication	Chrysanthemum and potato	[84]
3	Luffa sponge	Multiplication and rooting	Philodendron spp.	[79]
4	Paddy straw, jute, coir	Rooting	Nicotiana, Beta, Chenopodium, Tectona, Musa,	[80]
5	Coir	Microcorm production	Gladiolus	[85]
6	Sugarcane baggase	Rooting	Apple	[81]
7	Peat pellets	Rooting	Sunflower	[86]
		Multiplication	Terminalia, Celastrus, Feronia, Boswellia, Chlorophytum	[87]
8	Glass wool	Multiplication	Chrysanthemum	[84]
9	Rock wool	Shoot development	Eucalyptus citriodora	[88],[89]
			Spathiphyllum	[90]
10	Nylon cloth	Multiplication	Chrysanthemum	[84]
11	Polyurethane foams	Multiplication	Nicotiana and Vitis	[91]
			Gladiolus	[78]
12	Foam plastics	Adventitious root development	Rhododendron	[92]
13	Polyester squares	Multiplication	Musa	[93]
14	Polyester rafts	Multiplication	Anthurium	[94]
15	Florailite and vermiculite	Multiplication	Ipomoea batatas (sweet potato)	[95]
16	Polypropylene Membrane Rafts	Multiplication	Gladiolus	[78]

Conclusion

The main challenge with commercial tissue culture technology is its high manufacturing costs. The development of micropropagation procedures in the laboratory as a component of R&D programs should result in a viable technology suited for the mass production of desired clones. The established methodology that is accessible

for a species and the advantages and risks attached to it are key factors in determining whether commercialization is successful. In numerous economic plant species, *in vitro* propagation is limited by lack of contemporary techniques to overcome rigorous labour manipulation. Scaled-up unit cost of micropropagules can be decreased by employing creative and more affordable options mentioned above. In order to determine the efficacy of such methods, pilot-scale testing is required.

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