A Review on Low Cost Methods for *In Vitro* Micropropagation of Plant Through Tissue Culture Technique

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**Abstract**
Medicinal plants have been the subject of man’s curiosity since the time of human survival. A considerable majority of people of the world’s population still rely on the traditional medicine for their primary health care necessities. Currently demand of herbal medicines has been enhanced; and it is very difficult to fulfill the demand from field plants. Hence, *in vitro* propagation of plant by tissue culture can be used to fulfill the requirement of medicinal plants. The techniques applied in tissue culture are expensive; hence we try to summarize the data for low cost method for *in vitro* micropropagation of plant through tissue culture technique. This can help the scholar working in developing of tissue culture methods for *in vitro* propagation of medicinal plant.

**1 Introduction**
Plants have been an important source of medicine for thousands of years. Even today, the WHO estimates that up to 80 per cent of people still depend mainly on traditional remedies such as herbs for their medicines. Plants are also the source of many modern medicines. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from or modeled on plant substances.

Consumer demand for high quality medicinal herbs is increasing at a slow, but steady, rate and many of these herbs are harvested exclusively from stagnant to declining wild populations. In order for herb farmers to be successful in providing a cultivated supply of quality herbs for the increasing public demand, diverse propagation methods need to be explored and utilized to provide them with sufficient volumes of quality planting stock.

Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods. Micropropagation is a vegetative propagation of plant under aseptic conditions. Very small explants can be used for micropropagation, which is impossible with conventional technique. Thus the technique is very valuable when only limited tissue is available as explants. It can be used to produce disease-free plants by excluding disease-causing organisms during the propagation cycle. The major advantage of micropropagation is the extremely high multiplication rates. Therefore, this technique is highly suited for rapid multiplication of rare genotypes, and of plants having rare genotypes. Some plants with very small seeds, including most orchids, are most reliably grown from seed in sterile culture and excess material produced can often be stored over long periods.

An often-cited disadvantage of modern plant tissue culture methods is the relatively higher costs involved as compared to other methods. The need for low-cost plant tissue culture systems, applicable for micropropagation and *in vitro* conservation of plant genetic resources, has been emphasized to allow the large-scale application of such technology in developing countries.

The use of chemicals such as carbon sources, gelling agents, inorganic and organic supplements, and growth regulators in culture media, make this technique expensive. Sucrose is usually used as a source of carbon and agar as the gelling agent, and together they constitute the most expensive components of the culture media.

The medicinal plants are becoming rare and threatened with local extinction due to increasing deforestation, and conversion of land into industrial or residential area. The level of knowledge of cost-effective propagation of plant is not common and has slowed down domestication of many trees. The low cost propagation of plant is a solution for domestication and conservation of indigenous and threatened plant species. Hence, this review discusses the development of low cost technique for micropropagation of plant through tissue culture technique.
2 Cost effective approaches

Micropropagation technology is more expensive than the conventional methods of plant propagation, and requires several types of skills. It is a capital-intensive industry, and in some cases the unit cost per plant becomes unaffordable. During the early years of the technology, there were difficulties in selling tissue culture products because the conventional planting material was much cheaper. Now this problem has been addressed by inventing reliable and cost effective tissue culture methods without compromising on quality. The cost of the medium per liter was worked out with modifications in its components.

2.1 Alternatives to Carbon Sources

Sucrose is the most commonly used carbon source in the micropropagation of plants. Sucrose adds significantly to the media cost. Household sugar and other sugar (sugar cubes) sources can be used to reduce the cost of the medium. Sucrose made of cane sugar and contains 99.98% sucrose and 0.01% reducing sugar. Household sugar (crystalline sugar) made of cane syrup treated with SO₂ (sulfitation) or CO₂ (carbonation) and contain 96-97% sucrose and 0.75-1% reducing sugar. Sugar cubes made from grains of refined crystalline sugar, and contain 99.5% sucrose and 0.03% reducing sugar. It is considered to be higher quality than crystalline sugar.

2.2 Alternatives to Agar

Agar has been taken for gelling agent in tissue culture media as it is biologically inert and has high gel clarity, stability and resistance to digestion by plant enzymes. In spite of its exclusive use and advantages as a gelling agent, many reports on its adverse effects have been published over the years, which include batch to batch variability, presence of impurities which may have inhibitory effects on growth. Moreover agar happens to be the most expensive constituent of plant tissue culture media followed by sucrose. Additionally local accumulation of heat, hindrance of the availability of dissolved oxygen to the cultured plant parts in media, contamination of media through agar sticking to the neck of the culture vessels etc have also been reported to be other disadvantages of agar. Sometimes roots grown in agar gelled media often get damaged during washing prior to transplantation and remains of agar gelled medium in the roots call for unwanted bacterial and fungal contamination.

Prakash (1993) stated that gelling agent, agar which is usually added to increase media viscosity contributes 70% of the media costs. The high production costs in the micropropagation procedures can drastically be reduced if cheap and (or) reusable alternatives to highly expensive tissue culture grade purified agar could be employed without compromising the quality of regenerated plants.

Instead of agar glass bead can be as reusable and biologically inert alternative support matrix in liquid culture medium. The glass beads (1.5 mm diameter) were soaked overnight in chomic acid and washed with Teepol 1% and distilled water and dried in hot air oven prior to use. About 30 g glass beads were added as support matrix instead of agar to 30 mL of liquid medium per 250 mL culture flask to which six explants were inoculated.

Cheaper alternatives to agar include various types of starches and plant gum. Gelrite can be replaced with starch-Gelrite mixture. The use of laundry starch, potato starch and semolina in a ratio of 2:1:1 reduced the cost of gelling agent by 70-82%. However, the addition of such gelling agents to the medium also has some disadvantages. Some gelling agents contain inhibitory substances that hinder morphogenesis and reduce the growth rate of cultures.

Corn and potato starch significantly increased the number of shoots/explant over the control treatment which had 7 g/l of agar. The highest number of shoots/explant was achieved in medium with 50 or 60 g/l of PS + 1 g/l of agar. Media with 50, 60 g/l of PS or 60 g/l of CS and 50 g/l of CS + agar at 1 g/l significantly enhanced the percentage of dry weight.

Corn-starch (CS) as a gelling agent has been used along with low concentration of ‘Gelrite’ (0.5 g ‘Gelrite’ + 50.0 g CS /l) for the propagation of vegetative plants.

Smykalova et al found that the shoot proliferation *Humulus lupulus* was better on corn starch-medium than on agar. However, it became difficult to detect the contamination because the CS medium turned grayish-white.

Naik and Sarkar substituted agar on potato micropropagated medium with 13% of sago and found that the number of shoots and leaves and root length were significantly higher compared to the agar medium.

Similarly, usefulness of psyllium or isubgol, locust bean gum, a cheap gelling agent, and guar gum for plant tissue culture medium has also been established as a cheap gelling agent.

Tyagi RK et al tested isubgol as alternative gelling agent for in vitro culture of *Curcuma longa* L. non-significant differences were observed between media solidified with this gum and agar during multiplication.

Goncalves S and Romano A, describes the successful use of locust bean gum (LBG) as a gelling agent in combination with agar for shoot multiplication and rooting of carob tree and Iberian rose shoots. In this work the use of LBG as the only gelling agent was not possible because in this case the medium was only weakly solidified. To increase the firmness of LBG gelled medium, various combinations of gum and agar were examined.
Babbar SB et al used guar gum gelled media for in vitro seed germination of *Linum usitatissimum* and *Brassica juncea*. The media used for these were gelled with either guar gum (2, 3, or 4%) or agar (0.9%). Guar gum-gelled media, like agar media, supported all these morphogenetic responses. Rather, axillary shoot proliferation, rhizogenic and embryogenic responses were better on guar gum-gelled media than on agar media.

Neeru Jain & Shashi B. Babbar has been successfully used Gum katira as a gelling agent in tissue culture media for *in vitro* shoot formation and rooting in *Syzygium cuminii* and somatic embryogenesis in *Albizia lebbeck*. Media were gelled with either 3% gum or 0.9% agar. Viscosity of gum katira gelled medium was less than one-sixth of the viscosity of agar-gelled media. To increase the firmness of the gum katira-gelled medium, various combinations of agar (0.2–0.6%) and gum (1–3%) were used.

Jain RR and Babbar SB studied on Evaluation of Blends of Alternative Gelling Agents with Agar and Development of Xanthagar, A Gelling Mix, Suitable for Plant Tissue Culture Media. Isabgol, Xanthan gum and guar gum having a distinct cost advantage over agar, none is likely to be used as routinely as agar because of some inherent drawbacks. Isabgol and guar gum remain highly viscous even at high temperature and therefore, pose problem in adjustment of pH and dispensing of the medium to culture vessels. Because of their flowing nature even at room temperature, neither of these forms stable slants in culture tubes. Gum katira gelled media remain viscous liquid and therefore, cannot be used for overlaying of heavy explants. Xanthan gum media, though more viscous than gum katira gelled media, too remain in liquid state and are also incapable of forming stable slants.

To overcome these shortcomings by developing gelling mixes having one gelling agent from among the alternative gelling agents and agar. Xanthagar, a mix of xanthan gum and agar, possesses all desirable properties comparable to agar and offers a substantial cost benefit over agar.

Nowadays, commercial micropropagation labs are using low cost agar alternative in routine protocols. Even liquid medium and micropropagation in bioreactors which eliminate agar contributes 70% of the costs. Other researchers evaluated polyurethane foam, coconut coir and betel nut coir in liquid medium.

2.3 Alternatives to distilled water

Water is the main component of all plant tissue culture media. Usually in tissue culture research, distilled or doubled distilled and de-ionized water is used. Distilled water produced through electrical distillation is expensive. In some cases, alternative water sources can be used to lower the cost of the medium. Distilled water was replaced by tap autoclaved tap water. If tap water is free from heavy metals and contaminants, it can be substituted for distilled water.

2.4 Use of liquid media

The use of liquid media eliminates the need of agar. Suspension cultures without gelling agents are commonly used for culturing callus, cell clusters, buds and somatic embryos. Suspension systems allow greater contact between the explants and the medium. Moreover, agitation of such media reduces the diffusion gradient in the nutrient supply. The toxic metabolites exuding from the tissues are also dispersed effectively.

3 Alternative to conventional equipments

An alternative to reduce the unit cost of tissue culture micropropagation, autoclave can be replaced by pressure cooker. Contamination was not observed when the media and equipment were sterilised using a pressure cooker instead of an autoclave. Culture bottles were also replaced by jam jars.

4 Other methods

- The explants, obtained from the green house found to be easy to surface sterilize than field grown plants in term of the major problem of contamination, it reduces the maintenance cost.
- There was no detectable contamination when sodium hypochlorite and Tween 20 were substituted with commercial bleach (JIK) and ungral for sterilisation of explants. Again this shows that these alternative materials can be used successfully for sterilisation purposes. The use of commercial bleach also reduced cost of sterilization.
- Plantlets were successfully acclimatised using rice husks and then transplanted into the potted soil in the shade net. Eighty three percent of the plants survived during the acclimatisation procedure when rice husks were used compared to 80% using conventional approach (use of vermiculite). Rice husks which are available and free of charge in the rice growing areas therefore, be used as an alternative resource during acclimatisation to reduce costs.

5 Conclusions

The scientific survey has suggested that, *in vitro* propagation of plant can develop at low cost comparatively modern technology. In this review, we have attempted to present the prominent and the most recent findings on the low-cost methods used in plant tissue culture for the *in vitro* propagation of plant.
6 References


