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ECONOMIZING TISSUE CULTURE TECHNOLOGY THROUGH USE OF LOW COST ALTERNATIVES: A REVIEW

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The present paper review the significance of economizing tissue culture technology through use of low cost alternatives.

Keywords : Low cost alternatives; Tissue culture.

Introduction

To fully exploit tissue culture technology in favor of horticulture, agriculture, silviculture and forestry, there is an urgent need to find out some options to reduce the cost of plantlet production through micropropagation.

Low cost options should lower the cost of production through micropropagation without compromising the quality of plants. So many factors influence the cost of plantlet produced through tissue culture, such as manpower, electricity, chemical and glassware etc. The reduction in the production cost of micropropagules can be achieved by improving process efficiency and better utilization of resources. The composition of tissue culture media used for micropropagation has tremendous influence on production cost. Use of house hold sugar and alternatives for agar (gelling agents) can reduce the cost of production. According to Prakash[1], media chemicals cost less than 15% of plantlet production. Of the media components, the gelling agents contribute 77.80% of the cost while carbon source 21.55% of the cost. In this review the authors summaries the various aspects with respect to gelling agents and carbon sources.

Several research and development projects have been undertaken to improve the productivity of agricultural, horticultural and forest trees by the European Union under Co-operation in the Field of Scientific and Technical Research (COST). Under this program, coordinated and funded by the European Union, one of the primary aims has been to reduce micropropagation cost. For example, the objective of 'COST 843' action has been the innovation of low-cost plant propagation methods that enhance sustainable and competitive agriculture and forestry in Europe[2]. The high costs of labour of micropropagation are a major bottleneck in the EU to fully exploit *in vitro* culture technology. In the EU, labour currently accounts for 60-70% of the costs of the *in vitro* produced plants. In another program, the large-scale production and introduction of bamboo in the EU using tissue culture technology has been undertaken with the main objective of reducing the costs of micropropagation[3].

There are many factors, which influence the cost of tissue culture raised plantlets like labor cost (including skilled and unskilled), infra structure including facilities of electricity supply, culture maintenance and of acclimatization, cost of culture containers and cost of plugs, media components (gelling agents, plant growth regulators, micro and macro nutrients and carbon source) etc.

Cost per plantlet can be reduced by reducing electricity consumption by designing, such growth rooms, where sunlight provide light, without interfering optimum temperature and using efficient explant sterilization procedures, otherwise establishment of culture costs very high and. Use of low cost gelling agents and carbon source will also help in lowering the cost of plantlets. The composition of culture media used for shoot proliferation and rooting has a tremendous influence on production costs. The main components of most plant tissue culture media are mineral salts and sugar as carbon source and water. Other components may include organic supplements, growth regulators and gelling agents[4]. Proper choice of media and containers can reduce the cost of micropropagation. The replacement of expensive alternatives to gelling agents, use of cheaper carbon source and some other medium components can reduce costs of production.

Gelling agents

Gelling agents are usually added to the culture medium to increase its viscosity as a result of which media get solidified. This semi solid media provide support to the

Table 1. Different gelling agent and their solidifying concentration and cost per liter media.

Gelling agents	Amount % (w/v)	Cost of gelling agent/ l media	Reference
Difco Bacto agar	-	24.00	18
Gelrite	-	32.50	
Agar A	-	17.60	
Agar B	-	6.48	
Agar C	-	3.46	
Cornstarch	-	1.46	
Agarose	0.9	695.00	19
Agar (Difco Bacto	0.9	98.00	
Agar (Qualigen)	0.9	17.00	
Alginate	0.5-2.0	131 @ 2%	
Carrageenan	1.0	76.00	
Ficoll	10.0-14.0	27324 @ 14%	
Guar gum (HiMedia)	3.0-4.0	7.20 @ 3%	
Gum Katira	3.0	9.00	
Isabgol (Tel Brand)	3	7.00	
Phytagel	0.3-0.5	48@ 0.5%	
Starch (Tapioca)	10	3.00	
Starch (Tapioca)	10	0.14	20
Agar agar (HiMedia)	0.8	22.53	21
Sago	15	0.54	
Isabgol-husk (Deer Brand)	5	1.26	
Guar gum	5	0.20	
Cassava flour	8.0	-	22
Cassava + agar L	(8.0 + 0.35 respectively)	-	
Agar	0.7	-	
Agar (Qualigen)	0.9	17.00	23
Xanthan gum (Shree Krishna Pharmaceuticals, India)	1.0	5.00	

explants. Growth and development of explant is influenced by quality and quantity of the gelling agent in media. Several kinds of gelling agents are available in market like agar, gelrite, phytagel, agarose, gellan gum etc. Agar is the most frequently used gelling agent for preparation of most of the plant tissue culture media, because of the desirable characteristics of high gel clarity, stability and resistance to digestion by plant enzymes during use. Earlier agar was also thought to be biologically inert but later on a number of reports on its adverse effects have been published[6-8], including batch-to-batch variability, inhibition of growth, presence of impurities and impartment and impairment of vitrification.

According to Debergh[8], it contributes to the matrix potential, the relative humidity and affects the availability of water and dissolved substances in the culture containers. Various brands and grades of agar are differing in the amounts of impurities and gelling capacity. Agar is available in market with varying price, level of purity and gelling capacity. Which kind of the agar grade should be used, it depends on one's target and on the plant species. It is usually unnecessary to use high purity agar for large-scale micropropagation; cheaper brands of agar have been successfully used for industrial scale micropropagation[9]. To solidify the media lowest concentration of agar depends on its purity and brand. Usually 0.6-0.8% (w/v) agar is used to solidify the media.

The use of liquid media eliminates the need of agar. Other options include white flour, laundry starch, semolina, potato starch, rice powder and sago etc. 70-82% reduction in cost of gelling agent has been reported by Prakash[1], by using laundry starch, potato starch and semolina in a ratio of 2:1:1. A number of substitutes for agar have been tried out including, methylcellulose and alginate[10], starches from barley, corn, potato, rice and wheat, gellan gum and potato starch[11,12], microcrystal cellulose[13], isubgol[14], gelatin, pectin and a number of other support systems such as agitated liquid medium, filter

Table 2. Low cost option for sugar in medium.

Sugar type	Use	Reference
Refined white sugar (RWS)	Culture of zygotic embryos	46, 47
Unrefined light brown sugar	Culture of zygotic embryos	46
Unrefined brown sugar	Culture of zygotic embryos	46
Table Sugar	Multiplication of banana, potato, orchids, chrysanthemum; shoot regeneration and rooting of lentil, peanut, chickpea	48

paper, cotton wool, polyester fleece and glass beads.

Differences in the performance of agars and gelled media have been attributed to limited diffusion of medium components and water[6,15], impurities[16] and to differences in gel strength[8]. The National Research Development Corporation, India has listed low cost agar alternatives, which are worth evaluating for routine use in commercial micropropagation[17]. The low cost options to agar, agarose, and gellan gum have been listed in Table 1 with their cost in per liter of the media.

However, the addition of such gelling agents to the medium may have some disadvantages. Some gelling agents contain inhibitory substances that hinder morphogenesis[24] and reduce the growth rate of cultures. Sometimes toxic exudates from the cultured explants may take a longer time to diffuse. These gelling agents may influence availability of mineral ions and plant growth regulators due to adsorbance of these molecules. Use of cheaper alternatives to agar may give a dark color to media, which make it difficult to take observations regarding contamination and rooting. These low cost alternatives to agar may create problem during dispersion of media into culture vessels. Again these solidifying agents may take more time and energy to clean the culture containers.

Combination of 50.0 g/l corn starch with 0.5g/l gelrite have been used for the propagation of fruit trees, such as apple, pear and raspberry, banana and sugarcane, ginger and turmeric[25,26]. The corn starch-medium proved to be better for shoot proliferation than on agar. The cost of corn starch was $1.8/kg compared with $200/kg of agar. However, it became difficult to detect the contamination because the corn starch medium turned grayish-white.

Nene and Sheila[27] used tapioca obtained from tubers of cassava (*Manihot esulenta* Crantz.), for tobacco and chickpea culture. Rooting of chickpea was found to be better on tapioca with 66.7% than on agar with 40%. Addition of 80.0g/l tapioca starch to the MS medium was found to be a good substitute for 'Bacto-agar' for potato shoot-culture[28]. The results reported by Gebre and Sathyanarayana[29] show the possibility of using tapioca as an alternative cheaper gelling substance (40x cheaper than agar at equal concentration) in micropropagation of potato through production of plantlets or microtubers. According to Maliro and Lameck[22] cassava flour (even without processing into pure starch) can be a substitute to agar and improve the growth of shoots of *Uapaca kirkiana* and *Faidherbia albida.* In a system, where subculture is done at two weeks interval, there is no need of mixing agar with it. If the cassava flour can provide both the gelling and carbon source requirements in the medium then it can substantially reduce the medium cost.

Barley starch (60.0 g/l) has also been used for culturing potato-tuber discs, and for anther culture of barley[30,31]. Sago (obtained from the stem pith of *Metroxylon*) at 13% concentration was substituted for agar in MS medium for the multiplication of chrysanthemum through shoot tip culture. The number of shoots and leaves, and root length were significantly higher on sago than on agar[32]. The cost of sago is $0.5/kg.

Isubgol is the dried seed-husk of *Plantago ovata.* It is an alternative gelling agent because of its polysaccharidic and colloidal nature, good gelling ability, resistance to enzymatic activity and better clarity than agar in gelled form has the potential to become a universal gelling agent for plant tissue culture media. However, its higher melting point (70.6°C) necessitates adjusting of pH and dispensing quickly[33]. Isubgol at 3% in MS medium has been used for the propagation of chrysanthemum[14,32]. The cost of 'Isubgol' is about $4/kg.

Babbar *et al.*[34] has reported guar gum as a cheaper alternative to agar. Seed germination response of two species *Linum usitatissimum* and *Brassica juncea* was found to be similar on both guar gum gelled-medium and on agar gelled media. The axillary shoot proliferation on 2% and 3% guar gum-gelled media was significantly higher than on agar medium both in terms of number of

shoots per responding explant and their subsequent growth in *Crataeva nurvala*. For *in vitro* rooting response of microshoots of *Crataeva nurvala* on agar and only 4% guar gum-gelled media was not significantly different. However, the elongation of roots was much better on guar gum-gelled medium than on agar medium. Guar gum was better than agar as a gelling agent for differentiation of embryos from callus cultures of *Calliandra tweedi.*

Guar gum, being 8 to 80 times cheaper than agar and Difco bacto agar, respectively; would definitely be useful, particularly in the plant tissue culture industry. The source of this low cost gelling agent is *Cyamopsis tetragonoloba.* This plant is under cultivation practices and widely cultivated therefore, increased demands can be met without any fear of exploitation of the natural resource. This herbal product is biodegradable and poses no threat to the environment on being disposed of after use. However, like Isubgol, media gelled with guar gum require quick adjustment of pH and dispensing.

Carbon source

It is well known that the earbon source in the culture medium is an essential component of the medium as a source of energy and for maintaining the osmoticum[35,36]. Sometime sucrose has some distinct morphogenetic effects also. Generally sucrose is used as a source of energy for *in vitro* cultures because normally under tissue culture condition tissue remains non-photosynthetic. The highest dry weight of cell suspension culture of *Acer pseudoplatanus* was recorded, when sucrose concentration ranged from 4% to 6% in the media[37]. And similar results have been reported in suspension culture of *Pinus elliottii*[38]. Sucrose is not always most effective carbon source for shoot induction. Sorbitol has been found to be better than sucrose in *Malus robusta*[39], while dextrose was satisfactory substitute of sucrose in tumor cell culture of *Picea glauca*[40]. There are a few reports whereby glucose and/or fructose have been found to be better sources of carbon than sucrose for inducing adventitious shoots or axillary buds[35,36,41]. Sucrose was better than both glucose and fructose in inducing shoot organogenesis in *P. pinea*[42].

Hydrolysis of sucrose results into formation of glucose and fructose. This glucose enters into pentose phosphate pathway, into DNA synthesis etc and ultimately stimulates morphogenesis[43]. For induction of somatic embryogenesis high concentration of carbohydrates is used for osmotic effect. Mannitol is considered as metabolically inert osmoticum for some species. However, it is not always metabolically inert as in some plants it is produced photosynthetically, translocated and stored also[44]. Polyethylene glycol (PEG) is also used as osmoticum.

The carbohydrate requirement for rooting of shoots depends upon availability of auxins, nitrogen and light[37]. It has promoting effect during pre-meristemoid formation but impose inhibitory effect thereafter. The need of carbohydrate is species and stage specific.

Sucrose adds significantly to the media cost. Table sugar and other sucrose sources can be used to reduce the cost of the medium. Sugar available at grocery stores in market is sufficient-pure for micropropagation. For culturing ginger and turmeric, Household sugar (3%), Double refined sugar (3%) and Sugar crystals (3%) were suitable alternatives to laboratory grade sucrose but Sugarcane juice (10% v/v) resulted into drying of leaf tips[1]. Use of common sugar in place of laboratory grade sucrose reduces the cost of the medium from 78% to 87%. The cost of the local sugar was US$ 0.55/kg against the $40.0/kg for the imported sucrose. In Bangladesh, several laboratories have used locally available household sugar for culturing potato, banana, orchids, chrysanthemum, lentil, peanut, chickpea, medicinal plants and fruit trees. According to Prakash *et al.*[5] local sugar was found to be as good as the high-grade laboratory sugar for the multiplication of banana. Maple syrup (from *Acer saccharum)* has been used for the multiplication (50.0 g/l) and rooting (34.0 g/l) of cherry root stocks from nodal segment and shoot tips.

According to Endress[45] several compounds are used in plant tissue culture for cultivation of cells namely glucose, saccharose, glycerol, pentoses and uronic acid. There are some other sources of carbon, which are used less frequently such as lactose, galactose and non-refined carbohydrates like molasses, whey, potato starch and grain starch. These non refined carbohydrates are used as low cost alternatives to refined pure sucrose. Some alternatives to purified sucrose have been worked out during last decade (Table 2).

Conclusion

To fully exploit tissue culture technology in favor of horticulture, agriculture, silviculture and forestry, there is an urgent need to find out some options to reduce the cost of plantlet production through micropropagation. Of the media components, the gelling agents contribute 77.80% of the cost while carbon source 21.55% of the cost. Systematic efforts must be made in the direction of testing low cost media alternatives for established tissue culture based mass multiplication protocols of different plants. Once media is economically optimized, other aspects that add to the cost of tissue culture technology may be addressed.

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