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Optimization of Low-Cost Media for Plant Tissue Culture: A Step towards the Formulation of Economical Culture Medium

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Abstract

For the propagation of valuable planting material, low-cost *in vitro* culturing technology is mostly adopted in the field of agriculture, horticulture, forestry, and floriculture in many developing nations. Low-cost options should minimize production and at the same time maintain the original quality. Cost reduction is done in low-cost technology by enhancing process efficiency and better resource utilization. This paper aims to highlight some better cost-effective alternatives of highly expensive media components such as agar, sucrose, and water that cost more than 70-85 % of total production. Our study enlightened the use of wheat flour, laundry starch, semolina, sago, and isubgol instead of agar, the use of household sugar and other local sugar rather than analytical grade sucrose, and the use of tap water without any heavy metals and pollutants in place of sterile distilled water showed promising result when adapted for a different purpose in any *in vitro* plant propagation. Also, some other low-cost technology is mentioned other than nutrient media to lower the cost of propagation and enhance productivity.

Introduction

The technique of plant tissue culture involves the *in vitro* culturing of cells, tissues, and organs on an artificial nutrient medium aseptically under controlled physical, chemical, and environmental conditions. The unique ability of plant cells to develop into a complete plant while culturing *in vitro* forms the backbone of plant tissue culture. This unique property of plant cells was predicted by Haberlandt and called "cellular totipotency". In a multicellular organism, a cell differentiates after regulated division. It is a process of specializing cell functions. Isolated cells from differentiated tissues are generally non-dividing and quiescent; to show totipotency, the differentiation process has to be reversed (called de-differentiation) and repeated (called re-differentiation). In plants, even highly mature or differentiated cells tend to turn into meristematic states as long as they show viability and are totipotent. The concept of totipotency describes the remarkable developmental adaptability that distinguishes plant cells from most animal cells.

This field is widely known in the modern era for improving the quality of crops and has ample applications like *in vitro* clonal propagation, popularly known as micropropagation, to raise disease-free true-to-type clones, somaclonal variation as a source of genetic variability for processed new cultivars, microspore culture to produce haploids and reduce the breeding period, pollen culture to screen gametic variation, and endosperm culture to produce a triploid plant that is difficult to raise in vivo.

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The whole technique can be separated into two sub-classes to achieve a particular objective: (A) Quantitative improvement includes micro-propagation through the adventitious shoot or nodal segment, meristem culture, somatic embryogenesis, and callus culture, (B) Qualitative improvement includes anther/microspore culture, ovary/ovule culture, endosperm culture, and protoplast culture. The applied areas of plant biology such as embryogenesis, morphogenesis, genetic alteration, pathology, nutrition, clonal propagation, and pathogen-free plant production also coincide with the technique of plant tissue culture [1].

The physiological status and genotype of the donor plant, the type of explants used and the method used to disinfect them, the constituents of nutrient media (including macro-and micronutrients, organic supplements, carbon sources, amino acids, and vitamins) as well as plant growth regulators, and culture conditions such as temperature, pH, light, and humidity, all play a role in tissue culture success [2]. Proper optimization of these factors cannot only enhance the growth of explants but also improve proliferation and morphogenesis. Plants' *in vitro* growth and morphogenesis are significantly regulated by the culture medium's composition. Initially, tissue culture mediums originated from nutrient compositions that are utilized for whole plant culturing, such as White's root culture medium and Gautheret's callus culture medium which were based on Uspenski and Uspenski's medium for algae and Knop's salt solution, respectively [3].

Materials and Methods

Nutrient media used for the *in vitro* culture of plant cells are generally composed of inorganic nutrients, organic nutrients, carbon source, gelling agent, Plant Growth Regulators (PGR) and antibiotics.

Inorganic nutrients are of two types namely: (a) macro-nutrients [required in large amounts (> 0.5 m mole/l concentration) and contain nitrogen, phosphorous, potassium, calcium, magnesium, sulfur, etc (Table 1)]; and (b) micro-nutrients [required in trace amounts (< 0.5 m mole/l concentration) and contain manganese, copper, cobalt, boron, iron, molybdenum, zinc, iodine, etc (Table 2)].

Organic nutrients are mainly vitamins, amino acids, and certain undefined supplements. Small amounts of organic nutrients can improve the development and morphogenesis of plant tissue cultures. Depending on the species and genotype, different amounts of these chemicals are necessary for effective culture. Like animals, plants also require vitamins that are supplied from outside in case of *in vitro* culture. The vitamins most commonly used are thiamine (vitamin B1), pyridoxine (vitamin B6), nicotinic acid (niacin), and others, as well as myo-inositol, a sugar alcohol that plays an important role during cell division because it breaks down into ascorbic acid and pectin and is incorporated into phosphoinositides and phosphatidylinositol [4,5]. In comparison to inorganic nitrogen sources, amino acids supply plant cells with nitrogenous supplements that are easily digested by tissues and cells. Amino acid mixtures such as casein hydrolysate (0.25-1 mg/l), L- glutamine (8 mM), cysteine (10 mg/l), L-asparagine (100 mg/l), and L-tyrosine (100 mg/l) are frequently used as sources of organic nitrogen in culture media [3]. In the initial phase, 'undefined supplements' like coconut and corn milk, tomato juice, malt or yeast extract, and casein hydrolysate which can contribute vitamins, amino acids, and growth regulators to a culture medium, were used to compensate for the requirement of organic substances in tissue culture.

Plant cells are heterotrophic, i.e., they rely on an external source of carbon while culturing *in vitro*. The most advisable ***Carbon source*** in plant tissue culture is sucrose because it is quite stable, available, and economical. During autoclaving, it gets hydrolyzed into fructose and glucose, wherein later morpho-genetically triggers the formation of auxiliary buds and divaricate adventitious buds. Besides sucrose, other carbohydrates e.g. lactose, galactose, and maltose, are also utilized but are not much more effective than either sucrose or glucose. Sometimes, it is required to add ***antibiotics*** to the culture media to prevent contamination. Generally, kanamycin and streptomycin at low concentrations are utilized. Some studies suggest that antibiotics should not be used because they limit cell growth as well. The best alternative to antibiotics is PPM (Plant Preservative Mixture) because it protects the culture from any kind of contamination, doesn't have an inhibitory effect on callus growth, and is heat stable.

Plant growth regulators are those compounds which even in minute concentrations, can modify the overall growth and morphogenesis of plants. Unlike animal hormones, the synthesis of a plant growth regulator is frequently not restricted to a single tissue but can occur in a variety of tissues. They can be transferred to and act in distant tissues, and they frequently act at the synthesis site. Another characteristic of plant growth regulators is their lack of specificity; each one affects a diverse set of processes. Seven main classes of PGRs are used in plant tissue culture, namely auxins, cytokinins, gibberellins, abscisic acid, ethylene, polyamine, and jasmonic acid (see Table 3).

Plant material can be cultured either in a liquid medium (e.g. anther culture, cell suspension culture) or on a semi-solid medium. The method used will be determined by the type of culture and its objectives. A solidifying agent is added to the liquid media before autoclaving to make a semi-solid media. ***Gelling agents*** are typically polymers that harden after being autoclaved. Some widely used solidifying agents are: (a) **Agar**, is a mixture of polysaccharides, including the neutral polymer fraction, Agarose (gives strength to the gel), and the highly charged anionic polysaccharides agaropectins (provides viscosity [6]), obtained from red algae *Gelidium amansii*, has several advantages: (i) It doesn't react with media constituents; (ii) It isn't digested by plant enzymes and remains stable at all feasible incubation temperatures. It melts at a temperature range of 60-100 ^{0}C and solidifies at 45 ^{0}C. In typical tissue culture media, 0.8 % agar is needed. (b) **Agarose** which is a meshy polymeric chain of approximately 50-150 monosaccharide units, composed of 3, 6- anhydro-α-L (1-4) galatopyranose and β-D (1-3) galactopyranose, and is prepared by purifying agar to remove the agaropectin. This is essential when high gel strength is required, as in the case of single-cell or protoplast cultures. (c) **Gelatin** is used at a high concentration (10 %) with limited success because it melts at a low temperature (25 ^{0}C). (d) **Gelrite** is obtained from the bacterium *Pseudomonas elodea*. It's easy to make in a cold solution at room temperature, and it sets as a clear gel. Unlike agar, its strength is unaffected over a wide range of pH, but few plants show hyperhydricity due to the presence of freely available water.

The formulation of any medium depends on the requirements of the culture type. This is a very crucial step, and there isn't one ideal approach to forming suitable media. However, starting with three media having different salt concentrations, such as high salt concentration, medium salt concentration, and low salt concentration, is desirable to get a favorable response

from the culture. For certain species, the entire concentration of salts in the media worked well, while for others, lowering the salt level to 1/2 or 1/4 times the maximum concentration improved *in vitro* growth.

Basic media that are generally used in plant tissue culture for different purposes are: (A) *Murashige and Skoog (MS) medium*: Two scientists named Toshio Murashige and Folke K. Skoog created it in 1962. This medium blends inorganic nutrients, vitamins, and amino acids and is mainly used for callus culture, micropropagation, and inducing organogenesis. (B) *Linsmaier and Skoog (LS) medium*: It was invented by Linsmaier and Skoog in 1965. It has similar components as MS medium with the increased concentration of thiamine hypochlorite (0.4 mg/l instead of 0.1 mg/l) compensated for the absence of vitamins except for inositol and were used for the same objectives as the MS medium. (C) *Gamborg (B5) medium*: It was invented by O. L. Gamborg in 1968. It has a higher concentration of nitrate and potassium, with a lower concentration of ammonia as compared to MS, and is mainly used for protoplast culture. (D) *Nitsch and Nitsch (NN) medium*: It was developed by J. P. Nitsch in 1969. It has a high concentration of thiamine, biotin, and folic acid, and is mainly used for anther culture. (E) *White's medium*: It was developed by P. R. White in 1963. It has a high concentration of magnesium sulfate and a low concentration of salt, with a 19 % reduction in nitrate concentration as compared to MS, and is mainly used for the shoot and callus culture.

The components and corresponding quantities of each media are shown in Table 4.

Table 1: Different macronutrients with their required concentrations and role.

Components	Concentration (mM)	Role
Magnesium	1-3	Part of chlorophyll molecules & cofactor for many enzymes reactions
Calcium	1-3	Constituent of the cell wall helps to maintain the integrity of the membrane and cell signaling
Sulfur	1-3	Constituent of some proteins
Phosphorous	1-3	For cell division as well as in storage and transfer of energy, photosynthesis
Potassium	20-30	For normal cell division, for the synthesis of proteins, chlorophyll, and for nitrate reduction
Nitrogen	25-60	Constituent of the amino acids, proteins, certain hormones, and chlorophyll.

Table 2: Different micronutrients with their required concentrations and role.

Components	Concentration (µM)	Role
Copper	0.1	Involved in electron transfer reactions, a cofactor for some enzymes
Cobalt	0.1	Component of vitamin B12
Iron	1	Component of ferredoxin &involved in electron transfer
Molybdenum	1	Component of certain enzymes (e.g. nitrate reductase), a cofactor for some enzymes
Iodine	5	-
Zinc	5-30	Required for chlorophyll biosynthesis, a cofactor for certain enzymes
Manganese	20-90	Cofactor for certain enzymes
Boron	25-100	Stabilization of constituents of the cell wall.

Table 3: Different PGRs, their role, and examples.

PGRs	Role	Examples
Auxin	Stem & internodes enlargement, tropism, apical dominance, abscission, rooting, etc. In tissue cultures, it is used for cell division and root differentiation.	Indole acetic acid (IAA), indole butyric acid (IBA), naphthalene acetic acid (NAA), naphthoxyacetic acid (NOA), para- chlorophenoxyacetic acid (p- CPA), dichlorophenoxyacetic acid (2,4-D), trichloro-phenoxyacetic acid (2,4,5-T).
Cytokinin	Apical dominance modification, shoots differentiation, etc. In tissue culture, it is used for the division of cells and adventitious shoots differentiation from calli and organs, and for the release of axillary buds from apical dominance to promote shoot proliferation.	Benzyl amino purine (BAP), isopentenyl-adenine (2-ip), kinetin, thidiazuron and zeatin.
Gibberellin	Stimulate normal development of plantlets from *in vitro* formed adventive embryos, release seeds, somatic embryos, apical buds, and bulbs from dormancy, and inhibit adventitious root formation.	20 known gibberellins (GA_3 mostly used).
Abscisic acid	Maturation of somatic embryos, facilitation of acclimatization, bulb and tuber formation, and promotion of the development of dormancy.	-
Ethylene	Senescence of leaves and ripening of fruits.	-
Polyamine	Promotion of adventitious root and shoot, and promotion of somatic embryogenesis.	-
Jasmonic acid	Enhancement of tuber and bulb formation, and enhancement of meristem formation.	-

Table 4: Composition of media most frequently used in plant tissue culture.

Medium components ($mg.l^{1-}$)	MS	B5	White	LS	NN	Chu/N6
Macronutrients						
$Ca_3(PO_4)_2$						
NH_4NO_3	1650.0			400.0	720.0	
KNO_3	1900.0	2500.0	80.0		950.0	2830.0
$CaCl_2.2H_2O$	440.0	150.0		96.0	166.0	166.0
$MgSO_4.7H_2O$	370.0	246.5	750.0	370.0	185.0	185.0
KH_2PO_4	170.0			170.0	68.0	400.0
$(NH_4)_2SO_4$		134.0				463.0
$NaH_2PO_4.H_2O$		150.0	19.0			
$Ca(NO_3)_2.4H_2O$			300.0	556.0		
Na_2SO_4			200.0			
KCl			65.0			
K_2SO_4				990.0		
Micronutrients						
KI	0.83	0.75	0.75			0.8
H_3BO_3	6.20	3.0	1.5	6.2	10.0	1.6
$MnSO_4.4H_2O$	22.30		5.0		25.0	4.4
$MnSO_4.H_2O$		10.0		29.43		3.3
$ZnSO_4.7H_2O$	8.6	2.0	3.0	8.6	10.0	1.5
$Na_2MoO_4.2H_2O$	0.25	0.25		0.25	0.25	
$CuSO_4.5H_2O$	0.025	0.025		0.25	0.025	
$CoCl_2.6H_2O$	0.025	0.025				
$Co(NO_3)_2.6H_2O$						
Na_2EDTA	37.3	37.3		37.3	37.3	37.3
$FeSO_4.7H_2O$	27.8	27.8		27.8	27.8	27.8
$MnCl_2$						
$Fe(C_4H_4O_6)_3.2H_2O$						
Vitamins and other supplements						
Inositol	100.0	100.0		100.0	100.0	
Glycine	2.0		3.0	2.0	2.0	
Thiamine HCl	0.1	10.0	0.01	1.0	0.5	1.0
Pyridoxine HCl	0.5	1.0	0.01	0.5	0.5	0.5
Nicotinic acid	0.5	1.0	0.05	0.5	5.0	0.5
Ca-pantothenate			1.0			
Cysteine HCl			1.0			
Riboflavin						
Biotin					0.05	
Folic acid					0.5	
Carbon source (sucrose)	3 %	2 %	2 %	2 %	2 %	5 %

Table 5: Effect of agar alternatives on medium solidification and culture growth of ginger and turmeric [15].

Agar alternative	Effect on medium and culture
Wheat flour (10%)	Sloppy medium, growth of cultures poor
Wheat flour (8%)	Proper solidification, growth of cultures below average
Laundry starch (6%)	Proper solidification, growth of cultures average
Semolina (5%)	Sloppy medium, growth of cultures average

Potato powder (7%)	Sloppy medium, growth of cultures average
Laundry starch + Potato powder + Semolina (2:1:1)	Solidification, growth of cultures as good as on agar media
Rice powder (11%)	Sloppy medium, growth of cultures poor
Sago (7%)	Proper solidification, growth of cultures normal

Table 6: Low-cost matrices used for different purposes.

Option	Use	Reference
Cotton fiber	Callus maintenance and shoot organogenesis	(**23**, Khan, Personal communication)
Glass wool	Multiplication of chrysanthemum	(**21**)
Nylon cloth	Multiplication of chrysanthemum	(**21**)
Polystyrene foam	Multiplication of chrysanthemum	(**21**)
Glass beads	Multiplication of raspberry and white clover	(**25**, T. Brinks, Univ.Hannover; Personal communication)
Filter paper	Multiplication of chrysanthemum, potato	(**21**)

Table 7: Effect of alternatives to analytical grade sucrose on culture growth of ginger and turmeric [15].

Alternative	Effect
Household sugar (3%)	Healthy cultures
Double refined sugar (3%)	Healthy cultures
Sugar crystals (3%)	Healthy cultures
Sugarcane juice (10% v/v)	Drying of leaf tips
Sucrose LR grade (3%)	Healthy cultures

Table 8: Low-cost option for sugar in a different medium.

Sugar type	Use	Reference
Refined white sugar (RWS)	Culture of zygotic embryos	(**19, 26, 27**)
Unrefined brown sugar	Culture of zygotic embryos	(**26**)
Sugar maple syrup	Multiplication and rooting of cherry rootstock. Replace micro-nutrients and reduce macro-nutrients	T. Brinks, Univ. Hannover, Personal communication
Table Sugar	Multiplication of banana, potato, orchids, chrysanthemum; shoot regeneration and rooting of lentils, peanut, chickpea	(**28**)

Table 9: Comparison of MS medium with LBTM (cost-wise analysis).

Conventional MS medium components	Cost (in Rupees for 1 l)	LBTM composition	Cost (in Rupees for 1 l)	% cost reduction
Macro-nutrients				
Ammonium nitrate	7.2	Ammonium nitrate fertilizer	0.1	98.6
Calcium chloride	1.9	Calcium chloride fertilizer	0.01	99.4
Potassium nitrate	1.1	Potassium nitrate fertilizer	0.6	45.4
Magnesium sulfate	1.3	Magnesium sulfate fertilizer	0.02	98.4
Potassium dihydrogen phosphate	1.8	Single super phosphate	0.8	55.5
Micro-nutrients				
Potassium iodide	11.2	Potassium iodide(LR)	1.9	83.0
Boric oxide	3.7	Power B-Boran, Boric powder	2.8	24.3
Manganese sulfate	1.4	Manganese sulfate fertilizer	4.0	66.6
Zinc sulfate	4.2	Zinc sulfate fertilizer	1.42	72.7
Sodium molybdate	1.1	Adbor powder	0.30	75
Copper sulfate	0.04	Chelated fertilizer	0.01	70
Cobalt chloride	0.2	Grandular/ powder	0.06	63.7
Iron-source				
Ethylene diamine tetra acetic acid (EDTA)	5.8	Ethylene diamine tetra acetic acid (EDTA)	5.8	0
Ferrous sulfate	1.1	Ferrous sulfate fertilizer	0.002	93.1
Vitamins				
Myo-inositol Glycine Thiamine HCl Nicotinic acid Pyridoxine HCl	1.4 4.8 1.7 1.5 5.3	Becosules B-complex Tablets (containing Thiamine, Riboflavin, Pyridoxine HCl, Ascorbic acid, Biotin, Folic acid, Calcium Pantothenate and Niacinamide)	1.0	92
Carbon source				
Sucrose	14.46	White refined sugar	3.50	75.8
Solidifying agent				

Agar (analytical grade)	64.8	Agar-Agar (AR)	24.0	62.9
Total	**152.48**		**40.86**	**73.20 %**

Results and Discussion

Currently, there are approximately 250 commercial tissue culture laboratories in India, with an overall mass production of 600 million plantlets per annum [7]. The higher cost of the advanced media components is the only cons [8]. Most of the tissue culture nutrients mediums are optimized based on the type of culture, i.e., explants used, and not on cost. It is good to use these standard tissue culture mediums in the laboratories and research centres, but it advisable while we are looking for the scale-up process i.e. from laboratory to market due to competition with rivals, ensuring minimal investment, and chiefly focusing on the ultimate financial gains, and any type of loss or damage at high scale production. So there is a need for a low-cost plant tissue culture system. Our study aims to highlight the low-cost alternative components of tissue culture media without compromising the quality of the culture. Other media components, like inorganic nutrients, organic supplements, and growth regulators, are required in fewer amounts and cost less than 15 % of total production costs; hence they are considered relatively cheap, and their optimization at the industrial scale is tedious. The use of chemicals like solidifying agents (mostly agar), sources of carbon and energy (chiefly sucrose), and sterile distilled water (requires electrical distillation assembly) in culture media, makes this method expensive as they contribute 85 % of the production cost. However, low-cost alternatives are available to replace these costly agents.

Alternatives of gelling agent

The physical condition of the culture media has a notable impact on the culture's growth and the proliferation of shoots or roots. Gelling agents are commonly added to culture media to improve viscosity, allowing plant tissues and organs to remain above the nutrient media's surface. Many solidifying agents are adopted in plant culture media, like agar, agarose, and 'Gellan gum', and are traded under labels like 'Phytagel, Gelrite', and 'Gel-Gro'. It impacts the availability of water and dissolved chemicals in the culture vessels, as well as the matrix potential and humidity [9]. Agar comes in a variety of brands and grades, each with different levels of impurities and gelling capability. The price, performance, and content of agar brands vary greatly. The choice of agar brand in a certain system and for a given plant species is ultimately determined by actual use and experience. For large-scale micropropagation, high-purity agar is usually unneeded; cheaper brands of agar have been successfully tested for industrial-scale micropropagation [10]. Thereafter, the optimum concentration should be used for large-scale production. Low concentrations of gelling agents have huge advantages in addition to cost savings. A semi-solid media ensures that the growing explant and the medium are in proper contact. It promotes growth by allowing greater diffusion of media components and can be easily removed from plantlets before transfer to field conditions. On this basis, semi-solid media is often favored over solid media. Varieties of starches & plant gums are less expensive alternatives to agar [11,12]. Various types of starches and plant gums are less expensive alternatives to agar. Low-cost agar substitutes are worth considering for routine usage in commercial micropropagation, according to the National Research Development Corporation, India (NRDC, 2002). Gelrite can be switched with the starch-Gelrite solution. [13]. Agar is eliminated when liquid media is used. White flour (in various concentrations), laundry starch, potato starch, rice powder, semolina, and sago are among other options. The addition of such gelling chemicals to the medium, however, has certain drawbacks. Some gelling agents contain inhibitory chemicals that stifle morphogenesis and slow down culture growth [14]. For *in vitro* multiplication of ginger & turmeric, different combinations of gelling agents were experimented by Prakash (1993) [15] who reported that the use of laundry starch, potato powder, and semolina in a proportion of (2:1:1) reduced the cost of the solidifying agent by 70-80 % without altering the culture's quality.The possible agar alternatives and their effects on medium as well as culture are shown in Table 5.

Corn-Starch (CS) has been used as a solidifying agent in combination with a low concentration of 'Gelrite' to propagate fruit trees, like apples, pears and raspberry, bananas, sugarcane, ginger, and turmeric [16,17]. On corn starch medium, shoot proliferation was better as compared to agar. However, the CS medium had turned grayish-white; it was a tedious task to detect the contamination. The supplementation of 8 % tapioca starch to the MS medium for potato shoot culture was shown to be an acceptable substitute for 'Bacto-agar' [18]. For potato-tuber disc and barley anther culturing, barley starch at 60 g/l concentration has been successfully used [19,20]. Sago (isolated from Metroxylon) at 13% conc. was substituted for agar in conventional MS medium for the micropropagation of chrysanthemums. On sago, the number of shoots and leaves, as well as the length of the roots, were much higher than on agar [21]. The cost of sago is $0.5/kg. 'Isubgol', which costs about $4/kg, is a colloidal mucilaginous husk, primarily made up of pentosans (produced from the seeds of *Plantago ovata*), has strong gelling activity, and has reasonable clarity when gelled. The chrysanthemum culture has been done by applying it at a 3% concentration in MS medium [21,22].

Low-cost matrices

For propagation of calli, cell clusters, buds, and somaclones, suspension cultures without gelling agents are often utilized. Suspension systems allow the explant to have the best possible contact with the medium. For growing callus and propagating shoots of *Taxus, Agrotis*, and *Artemisia*, sterilized non-chlorine bleached, rolled cotton fibre has been successfully used [23]. Cotton fibre acts as a support for growing plants in suspension medium and has recently been reported in commercial orchid, banana, chrysanthemum, and potato growth in Pakistan and Bangladesh. Banana shoots grew quickly in cotton-fiber and liquid MS medium and could be subcultured in two weeks instead of six (S. Khan, personal communication). On cotton, orchid protocorm initiation and shoot development were substantially faster than on agar-based media. The cost of cotton fibre is about $2.5/kg, while agar is $100- 200/kg depending on the manufacturer. Other alternative culture supports include foamplastic, bridges of filter paper, glass beads, 'Viscose' sponge, wool of glass, and rock in liquid media [24]. The possible alternative low-cost matrices and their uses are shown in Table 6.

Alternatives of carbon source

Sucrose is the most common carbon source utilized in the *in vitro* propagation of plants. Sucrose adds significantly to the media cost. The cost of the medium can be reduced by using household sugar and other sugar sources. Sugar available in su-

permarkets is pure enough for micropropagation. The cost of the medium is reduced by 78 to 87 % when common sugar is used. The cost of the local sugar was US$ 0.58/kg against the $40.0/kg for the imported sucrose. The possible alternatives to analytical sucrose for the micropropagation of ginger and turmeric and their effects on the same are mentioned in Table 7. The possible substitutes for sucrose and their uses for *in vitro* plant propagation are shown in Table 8.

Low-cost alternatives of sterile distilled water

All plant tissue culture mediums contain water as the primary component. Water that has been distilled or double distilled and de-ionized is commonly utilized in tissue culture research. The cost of distilled water produced by electrical distillation is significantly high. Alternative water sources can be employed to reduce the expense of the medium in some instances. Tap water can be used instead of distilled water if it is devoid of heavy metals and pollutants. It is successfully utilized for the tissue culture of bananas [29] and ginger [30]. A low-cost solution is to use table bottled water from the supermarket, but its mineral composition should be considered as it may influence pH and nutrient intake (H.J. Jacobsen, University of Hannover, Personal communication).

Majuja et al. (2017) [31] tested the effect of using low-cost alternatives on the micropropagation of bananas (*Musa* spp. L.). They have replaced conventional components of nutrient media such as analytical grade sucrose with household sugar and sugarcane juice; agar with isubgol, sago, corn starch, and cassava starch, and distilled water with autoclaved tap water devoid of any heavy metals and contaminants. The experiments showed a positive response and cost reduction was then analyzed. The total % cost savings while using household sugar and sugarcane juice as carbon sources was 96 %; isubgol, sago, corn starch, and cassava starch as the gelling agent was 92.5 %; and autoclaved tap water instead of distilled water was 98 %. Overall, 90 % cost of propagating bananas *in vitro* was reduced by replacing standard media components with locally available substitutes. Therefore, it is suggested that these low-cost alternatives can be adopted for the mass multiplication of bananas. Another experiment was done by Dhanalakshmi and Stephan (2014) [32]. They used a Low-Cost Banana Tissue Culture Medium (LBTM) for micropropagation of banana (*Musa paradisiaca* L) through shoot-tip culture (Table 9). Overall, 73.20 % of the cost of banana propagation *in vitro* was reduced by replacing standard MS media components with low-cost banana tissue culture medium. Therefore, it is suggested that LBTM can be utilized for *in vitro* multiplication of bananas. Along with that, they have replaced conventional instruments with low-cost instruments for banana tissue culture, and the price was compared to check the % cost reduction. Among them, the laminar airflow chamber with all accessories was substituted with the laminar airflow chamber with the minimum accessories; the autoclave was replaced with an ordinary pressure cooker; the air conditioner was replaced with a window air conditioner, etc., and it was reported that a total of 84.31 % cost was reduced.

Plant tissue culture is a capital-intensive method, and the unit cost per plantlet might become expensive in some situations. Hence, solutions to lower production costs must be adopted. The adoption of possible cost-effective strategies and the use of low-cost equipment to reduce the unit cost of micropropagules are considered low-cost tissue culture technology. The equipment and buildings with a preparation room, inoculation room, culture room, hardening and weaning area, polyhouse (greenhouses, plastic tunnels), packaging and shipping area, and related facilities such as an office, and a store for chemicals, containers, and supplies are the basic physical equipment of any plant tissue culture facility. The physical components of a tissue culture facility will vary in size according to the functional needs, i.e., the volume of production. Careful planning of a facility can result in significant cost reductions in both development and day-to-day operations. Before setting up a new facility, it is advisable to visit an existing laboratory to see the layout and operational requirements. Propagation costs can be decreased by selecting appropriate culture media and containers. The composition of media utilized for propagation has a significant impact on production costs. The type of culture vessel has an impact on the effectiveness of transfer during subculture and propagule generation. The mass making of media and its storage as deep-frozen stocks also lowers labor costs. Changing the artificial illumination to natural light is a good low-cost option in tissue culture. Air conditioners that are used to maintain a particular temperature for *in vitro* culture don't directly contribute to culture growth but at the same time add to the overall cost. Most *in vitro* grown plants can tolerate wide fluctuations in temperature, and adapt better to ex-vivo conditions than to *in vitro* conditions. Plants are sturdy and withstand transplantation when hardened in open shade under natural light. The multiplication and growth rate of cultures can be enhanced in a bioreactor which requires less space, energy, and labor. However, the use of bioreactors requires indexed plant cultures, and aseptic practices during the handling of plant material; otherwise, culture contamination leads to enormous economic loss.

Conclusion

Our study elaborates on some better low-cost alternatives to conventional plant tissue culture medium. The necessity for low-cost plant tissue culture systems that may be used for micropropagation and *in vitro* conservation of plant genetic resources has been highlighted to enable large-scale implementation and adaptation of such technology in developing nations (IAEA, 2004). These cheap alternatives shouldn't compromise the quality of micropropagation. [32]. Hence, the adoption of these alternatives for *in vitro* propagation would reduce production costs significantly, leading to an expansion in the area grown with tissue culture-raised plantlets.

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Compliance with ethical standards

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