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Low Cost Tissue Culture Technologies in Vegetables: A Review

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Authors' contributions

This work was carried out in collaboration among all authors. Author RN write the first draft of manuscript, author AB conceived the paper and write/edited the paper, author RKG finalize the draft and overall supervise all the aspects, authors AW and AG managed the literature searches. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

The demand of vegetable crops is increasing day by day due to changes in consumption patterns, so the need of the hour is to develop technologies that enhance the vegetable production at a rapid rate. Plant Tissue culture is one such remarkable biotechnological tool that has its application in vegetable propagation and improvement, disease elimination, herbicide resistance, salinity tolerance, incorporation of high nutrient content, genetically improved plants and conservation of endangered plant species and in the near future usage of this technology is going to increase further manifold. It is used for production of disease free quality planting material and development of varieties through direct regeneration, anther/ovule culture, somatic embryogenesis etc. or for creation of new variation (organogenesis *via* callus formation, soma-clonal variation and *in vitro* mutagenesis). In spite of being a very important and viable non-conventional biotechnological tool, high cost of production of seedlings *in vitro* remains a major impediment in popularization of this technology. High cost of producing seedlings is due to availability of limited resources, high recurrent costs of consumables for media and lack of awareness, which limits its application only to a few institutions and rich farmers especially in developing countries. Therefore, in order to make this technology a successful and viable option for the farmers, future thrust must be on cost

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reduction of *in vitro* seedlings. The components of tissue culture technology such as culture media components, glassware, lighting and water for media preparation can be replaced with low cost alternatives to reduce the overall cost of tissue culture. The usage of alternatives for gelling agent's like isabgol (potato, tomato, cassava, turmeric, ginger), sago (potato, tomato, turmeric, ginger) cassava starch (potato, cassava, sweet potato) barley starch, phytagel etc. and for carbon sources like table sugar (potato, turmeric, ginger), jaggery, sugarcane juice, cube sugar (bittergord), brown sugar etc have already been documented worldwide. The present paper reviews the work done by researchers around the globe in developing various low cost alternative technologies with focus on vegetable crops.

Keywords: Low cost tissue culture; gelling agent alternatives; natural lighting; low cost media.

1. INTRODUCTION

Plant tissue culture is the *in vitro* aseptic culture of cells, tissues, organs or whole plant under controlled nutritional and environmental conditions often to produce the clones of plants [1]. The controlled conditions provide the culture an environment conducive for its growth and multiplication, these conditions include proper supply of nutrients, pH medium, adequate temperature and proper gaseous and liquid environment. The theoretical basis for plant tissue culture was proposed by Gottlieb Haberlandt in his address to the German Academy of Science in 1902 on his experiments on the culture of single cells. He established the concept of totipotency, and further indicated that the technique of cultivating isolated plant cells in nutrient solution permits the investigation of important problems from a new experimental approach [2]. Plant tissue culture technology is being widely used for large scale plant multiplication and apart from their use as a tool of research, plant tissue culture techniques have in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. Small pieces of tissue (named explants) can be used to produce hundreds and thousands of plants in a continuous process. A single explant can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis [3]. Endangered, threatened and rare species have successfully been grown and conserved by micropropagation because of high coefficient of multiplication and small demands on number of initial plants and space.

In vegetable production and improvement, tissue culture is playing its fair share of role, micropropagation is a very useful tool for large scale production of disease free planting material in many vegetables especially potato. Plant tissue culture combined with recombinant DNA technology are the essential requirements for developing transgenic plants. Culture techniques like anther/pollen or ovule culture, meristem culture can themselves be utilized for crop improvement or may serve as an aid to conventional plant breeding. Protoplast fusion technology is useful for the purpose of interspecific hybridization where it is not possible using conventional methods. Mitochondrial recombination occurring after protoplast fusion has been of practical usefulness for the elimination of unfavorable traits resulting from nuclear cytoplasmic incompatibility after inter-specific hybridization. There are successful examples of such genome transfers in *Brassica*, *Cichorium* and *Lycopersicon*. In cabbage, male sterile cybrids are being utilized by seed companies in France to produce hybrid seeds on commercial scale and at competitive rates. Plant tissue and cell culture also provide germplasm storage options as cryopreservation of cell or embryo culture and low temperature storage of organized tissue. Tissue culture applications and its importance are increasing and it is likely to grow more in the future.

High production cost has been an impediment to tissue culture adoption which has further limited the technology to a few institutions and rich farmers while locking out the resource-challenged subsistence farmers. One factor contributing to the high cost of production is the cost of the culture nutrient medium which requires chemicals that are often very expensive [4]. By utilizing low cost alternatives we can reduce the cost of tissue culture greatly so that it can be practiced by an average farmer. The production cost of tissue culture plants can be reduced by 50-90 percent by using low cost alternatives [5].

Appreciable literature or knowledge generated by different researchers on low cost tissue culture of

vegetables have been published which are spread in various forms such as review articles, chapters in books, books, bulletins, catalogues, scientific journals, popular magazines etc. It is difficult for all researchers, teachers, students, amateurs, commercial growers, business houses, nurserymen and farmers to get an overview of earlier and recent developments regarding low cost tissue culture. Attempt has been made in this article to put together important information to develop a complete documentation of the results of the research and demonstrations conducted by different scientists on low cost tissue culture of vegetables. The document has been prepared only from published information as a review article and an attempt has been made to cite maximum important publications suggesting cost reduction in tissue culture of vegetables. The main objective of the review is to create awareness among the plant tissue culture utilizing community to make the technologies simple.

2. NEED FOR LOW COST TISSUE CULTURE TECHNOLOGY

Commercial application of tissue culture technology is restricted due to high production cost [6,7]. Hence, the most challenging aspect at present is to reduce the production cost, thereby improving the production efficiency [4,8,9,10,11, 12,13,14,15]. Micropropagation protocols have been developed for many vegetables but due to high production costs only a limited number are being produced on large scale through micropropagation and to overcome this limitation, numerous low cost strategies have been developed worldwide. Low cost tissue culture is very useful not only for farmers but also for routine large scale commercial multiplication [16]. Different plant tissue culturing components are, namely, nutrients/ media chemicals (plant growth hormones, vitamins and minerals nutrients), plant materials, equipments (culture containers, autoclave, laminar flow, instruments used for micropropagation, pH meter etc) and the infrastructures (media preparation, inoculation, growth and hardening rooms) and all these factors are subjected to play important roles in cost reduction [17].

3. ADOPTION OF LOW COST OPTIONS

The adoption of wrong low-cost options may make the production process prone to disasters. Low cost tissue culture techniques are more likely to succeed only if the basic conditions for tissue culture are scrupulously adhered to maintain propagules quality. It is the procedure followed which ensures the quality of tissue cultured plants not its sophistication. Low cost tissue culture technology means an advanced generation technology, in which cost reduction is achieved by improving process efficiency, and better utilization of resources [18].

Low cost options should lower the cost of production without compromising the quality of the micro-propagules and plants [19]. The primary application of micropropagation has been to produce high quality planting material, which in turn leads to increased productivity in agriculture. The generated plants must be vigorous and capable of being successfully transplanted in the field, and must have high field survival. In addition, they should be genetically uniform, free from diseases and viruses, and price competitive to the plants produced through conventional methods. Reducing the cost should not result in high contamination of cultures or give plants with poor field performance [4].

The foremost requirement of micropropagation is the aseptic culture and multiplication of plant material [20]. Microbe-free conditions need to be maintained in many cases, mistakes in concept or practice can introduce microbes in the culture containers from an external source or the plant material itself (endophytic contamination) and as a result, the microbes overgrow the cultures, and wipe them out. To prevent that aseptic conditions need to be maintained in culture containers, and during successive subcultures as microbial contamination of cultures is known to wipe out work of months, and can turn into a nightmare. The best low-cost option is to discard and dispose of contaminated cultures outright. Avoiding contamination in small R&D laboratories is not a difficult task where only a low number of cultures are handled. However, commercial production involves handling of thousands of cultures each day, so it is essential to maintain such cultures in large numbers under contamination-free conditions, until they are used for either further subculture or hardening and growing-on.

4. LOW COST TISSUE CULTURE TECHNOLOGIES

Low-cost tissue culture technology is the adoption of practices and use of equipment to reduce the unit cost of micro-propagules and plant production. A number of low-cost alternatives can be used to simplify various operations and reduce the costs in a tissue

culture facility, that's why proper choice of media and containers can reduce the cost of micropropagation [21]. There are many ways to reduce the cost of tissue culture production technology and one should always be careful to increase efficiency of production while reducing the overall cost. Various operations can be simplified and cost may be reduced by adopting low-cost alternatives [8,22,23].

4.1 Low Cost Washing and Sterilizing Operations

The washing cost of containers can be reduced by washing them manually instead of using costly machines and then they can be dried in the sun. The autoclaves used for sterilization operations are very expensive so these can be replaced by pressure cookers by placing a wire mesh at their base [24]. Contamination are not detected when media and equipments were sterilized using a pressure cooker. Costly aluminium foil is generally used for wrapping the instruments before sterilization which can be substituted by autoclavable stainless steel containers.

4.2 Low Cost Gelling Agent Alternatives for Vegetable Tissue Culture

Agar was introduced as a gelling agent more than 100 years ago and since then it has been extensively used as for microbial and plant tissue culture media [7]. It is useful for the purpose of culturing due to its stability, high clarity, nontoxic nature and resistance to its metabolism. In the recent past several attempts have been made to look for suitable substrata that could possibly replace agar in culture medium because of doubts about its inertness and non-toxic nature, fear of over-exploration of its sources and above all, the high cost of tissue culture grade agar [7,25]. It is the most expensive constituent of plant tissue culture media and it is reported that agar, which is usually added to increase media viscosity contributes 70% of the media costs [26]. In the recent past agarose [27], alginates [28], gelrite [29], isubgol [25,30], xanthan gum [7], guar gum [31,32], starch [33,34] have been used with reasonable success as substitutes for agar.

4.3 Use of Liquid Media and Physical Matrices

Agar can also be substituted by liquid media and physical matrices. Suspension cultures without gelling agents are commonly used for culturing callus, cell clusters, buds and somatic embryos. In suspension systems, there is greater contact between the explant and the medium, the agitation of such media reduces the diffusion gradient in the nutrient supply and also the toxic metabolites exuding from the tissues are also dispersed effectively. The liquid-media also have some disadvantages like damage to the delicate tissue during agitation. In some species, shoots submerged in liquid media become hyperhydric (water soaked), and unsuitable for micropropagation [35].

4.4 Low Cost Nutrient Alternatives

Plant tissue culture media contains macronutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) for better growth. The important micronutrients for plant tissue growth include iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu) and molybdenum (Mo). Among all the micronutrients iron is usually the most critical of all the micronutrients.

Locally available fertilizers in appropriate concentrations can be used as low cost source of nutrients for tissue culture. The conventional sources of MS media can be replaced by mixed nutrients containing both macro and micronutrients. For the micropropagation of cassava, fully substituted media with commercially available nutrients (Hydro Agri's fertilizer) was used resulting in reducing cost by 93.1% as compared to the traditional media [36]. Different kinds of fertilizers at different concentrations were tried which resulted in cost reduction of 24.4 percent for the medium prepared [37]. The possibility of using locally available fertilizers as alternative nutrient sources for cassava micropropagation was evaluated [38] and a low cost protocol for cassava tissue culture using Easygro vegetative fertilizer as an alternative source for conventional MS salts was also developed [38]. The effect of using low cost macronutrient substitutes- Ammonium fertilizers, potassium fertilizers and epsom salt on i*n vitro* regeneration of sweet potato has also been studied [39]. It was found that the Epsom salt substituted media, performed better in regeneration in term of leaves and nodes formed compared to the conventional media, while in other substitutes significant differences were not detected. It was observed that the fertilizer based medium was able to stimulate root formation in potato *in vitro* micropropagation culture just as much as the MS medium and in terms of plant

height, no significant difference was recorded [40].

4.5 Low Cost Carbon and Energy Alternatives

The most common carbon source in the micropropagation is sucrose and it has been reported as a source of both carbon and energy [41] but it adds greatly to the media cost. The carbon source such as grade sucrose that is often used in the micropropagation of plants at laboratory contributes about 34 percent of the production cost [42] Household sugar and other sugar sources have been used for culturing ginger, turmeric, potato, banana, orchids, chrysanthemum, lentil, peanut, chickpea, medicinal plants, fruit trees etc. to reduce the cost of the medium [5,26]. Use of common sugar as a substitute reduces the cost of the medium between 78 to 87% also the sugar sold in grocery stores is sufficiently pure for micropropagation.

For culturing ginger and turmeric, all other carbohydrates except sugarcane juice, were suitable alternatives to laboratory grade sucrose [26]. The alternative cheap sources of carbon and energy in potato culture media in order to reduce the overall cost of micro-propagation were also evaluated [42,43]. They used laboratory grade sucrose with two types of local commercial table sugar (white and brown sugar). Brown sugar enhanced significantly higher mean number of roots per plantlet after four subculture generations for all cultivars. Results also showed that table sugar not only enhanced micro-propagation but also significantly lowered the production input costs by 34 to 51 percent when compared with the analytical sucrose. Table sugar was used as a low cost alternative medium component for commercial propagation of potato and it was concluded that 97% cost can be reduced by using table sugar as carbon sources [44,45]. Up to 73 percent decrease in cost of media for plant regeneration and in vitro conservation was achieved in *Curcuma longa* cv. Prathibha by using inexpensive carbon source and gelling agent [46].

The carbon source in plant cell suspension culture is a very important factor for growth and development. Role of different carbon sources such as analytical grade sucrose, commercial grade sugar, sugar cubes and jaggery on cell growth of *Momordica charantia* has been reported and it was observed that significant improvement in cell growth occurred on a medium containing sugar cubes [47]. Similarly, maximum shoots per explants, shoot and root length and roots per shoot were obtained on MS medium containing ordinary sugar as compared to MS medium supplemented with analytical grade sucrose in ginger [48]. It concluded that there was no significant difference in response of *in vitro* cultures of jackfruit for shoot proliferation when carbon source was supplied either as analytical grade sucrose or as market grade table sugar [49].

5. APPLICATION OF LOW COST GELLING AGENT ALTERNATIVES FOR *IN VITRO* CULTURE IN VEGETABLES

5.1 Potato

Tapioca starch (8%) has been used as a good substitute for 'Bact-agar' for potato shoot culture [50,51]. Barley starch (60 g/l) can also be used for culturing potato-tuber discs [52], [53]. Sago, a processed (gelatinized) edible starch, was successfully used as a gelling agent in culture medium for *in vitro* potato culture [54]. Gelrite/Phytagel reportedly gave better results when used as gelling agent than agar in potato *in vitro* micropropagation [55]. Although the unit cost of gelrite/phytagel is more than agar but the quantity used for solidifying unit quantity of media is much less (25%) and it leads to save 43 to 52% cost on gelling agent. It has also been reported that gelrite based media have more ash content and water availability than agar [56]. Isabgol as a potential gelling agent for *in vitro* micropropagation of virus free potato was also evaluated [57] and from this study it was reported that Isabgol @12 g/l was the most suitable concentration and its performance was at par with agar solidified media for *in vitro* micropropagation of virus free potatoes. Corn and potato starch was used as an agar alternative for *Solanum tuberosum* micro-propagation and the highest number of shoots (6.8) was achieved in medium with 50 or 60 g/l of PS + 1 g/l of agar [58]. A cost-effective protocol for micropropagation of potato by using Balanga (*Lallemantia royleana*) seeds (45 g/l) as gelling agent for micropropagation of potato was established [59], their results indicated that balanga seeds show parallel even better results than agar to some extent and it is about 32% cheaper than agar.

5.2 Ginger and Turmeric

Isabgol produced maximum no. of shoots per culture when used as substitute gelling media for ginger in vitro micropropagation [60]. Agar was substituted by isabgol as gelling agent in *Curcuma longa* cv. Prathibha in vitro culture and no adverse effects on shoot regeneration and conservation were observed on isabgol-gelled low cost media [46]. Shoot bud explants of *C. longa* cv. Sona were cultured on modified MS media supplemented with 2.5 mg l^{-1} BAP + 3% sucrose and six gelling agents viz. 7 g l^{-1} Agar, 2.5g l^{-1} Clarigel, 4.5 g l^{-1} Clarigar, 6 g l^{-1} Gelzen, Isabgol 3.5 g l^{-1} and 2.5 g l^{-1} Phytagel [61]. Highest rate of shoot multiplication was recorded with 2.88± 0.03 shoots/ explant in the media solidified with Clarigar compared to regularly used gelling agent Agar (2.31± 0.38).

5.3 Tomato

The effect of low cost gelling agent's viz., isabgol and sago was studied as compared to agar on shoot multiplication in six tomato genotypes [62]. The results revealed isabgol to be the most suitable gelling agent recording maximum shoot multiplication in all the genotypes.

5.4 Cassava

A low cost medium for cassava was developed and the results revealed that the medium which had cassava starch 10% (w/w) as gelling agent showed generation ability comparable to the conventional media [36]. It was reported that among all the different media alternatives tested for cassava *in vitro* culture mean shoot height similar to that of conventional media was observed in the media containing agar agar strip (14 g/l) + corn starch(20 g/l) as gelling agent, 100 ml coconut water as growth regulator, 30 g table sugar as carbon source and 2 ml/l maxigreen 50 liquid fertilizer as substitute for MS salts [63]. Cassava was micro-propagated using enset starch (*Ensete ventricosum* Cheesman) as an alternative gelling agent which revealed that the concentration 80 gm/l bulla alone reduces 86% of cost, while composite of bulla 60 gm/l and 70 gm/l with agar 2 gm/l and 1 gm/l respectively saved 65-75% cost of gelling agent in plant tissue culture media [64]. Besides substituting conventional agar, bulla enhances root length compared to conventional agar which might be due to carbon ingredient found within it. However bulla showed poor clarity that caused difficulty in detecting contamination.

5.5 Sweet Potato

Starch extracts from cassava, sweet potato and Irish potato were tested as cheap alternating gelling agents for micropropagation of sweet potato and of the three sources of starch sweet potato gave the highest multiplication rate and it was also the cheapest [65]. A gelling agent, a mixture of starch from seeds of pigeonpea (*Cajanus cajan*) and cassava starch (*Manihot esculenta*) developed in Brazil (patent PI9003880-0 FAPESP/UNESP) was tested as an alternative to agar in the micropropagation of sweet potato (*Ipomoea batata*), the medium represents a good alternative to agar and the reduction in the final cost of culture medium was over 94% [66]. MS basal media gelled with agar, gelrite and different cassava starches; supplemented with 30 g/l sucrose was investigated for *in vitro* propagation of sweet potato plantlets [67]. Highest shoot height, leaves and node increases of 10.7±2.5 cm, 15.5±1.4 and 14.5±1.6 were obtained from TMS 98/0505, TMS 92/0057 and TMS 92/00057 newly processed starch-gelled media compared to 13.6±2.7 cm, 19±2.1 and 17.7±2.0 from gelrite-gelled media after 8 weeks of culture.

5.6 Wild Carrot

Wild carrot cultured by using corn starch as gelling agent showed higher yield of anthocyanin and dry weight of embryos [68]. The starch-mediated increase in growth and differentiation of wild carrot cells was accompanied by an increase in density of the cultures shown by higher dry weight/fresh weight ratios.

5.7 Garlic

Stimufol® was used as an alternative to the most widely used MS media [69]. It was used at levels of 0.0, 0.5, 1, 1.5 and 2 g / L^{-1}. Further, Assiutmix1, a product that makes the medium self-sterilized at 3.5 mg/L^{-1} was added to the medium. The statistical analysis indicated that both the MS and the Stimufol® have the same effect on the growth of garlic. This means that it can be used for a good and inexpensive alternative to basal medium (MS).

5.8 Radish

The potential of alternative cheap gelling agents was examined (*corn* flour, *kithul* flour, *barley*, *saw*, *wheat* flour and *undu* flour) for seed germination of radish (*Raphanus sativus* L.) var.

Beeralu Rabu [70]. Seed germination was observed on MS basal medium supplemented with different alternative gelling agents (10%) and agar (0.8%). It was found that agar and alternative gelling agents successfully produced plantlets from the seed explants of radish after 4 weeks and mean height, weight and number of seeds germinated in MS media with corn flour were not significantly different from agar. Cost of gelling agent was reduced in 95% by using corn flour as solidification agent instead of agar.

6. AUTOTROPHIC MICROPROPAGATION

Plants with functional chloroplasts can grow *in vitro* on media without sugar, provided the micropropagation environment is modified to enable photosynthesis. The growth of plants on sugar-free medium, but with the carbon dioxide enriched environment was similar to sugar-containing media [71]. This technique is termed as PTCS-'Photoautotrophic Tissue Culture System'. In the autotrophic system, plants are grown in large containers where the air content (oxygen, carbon dioxide, relative humidity, etc) and the composition of the culture medium is easily controlled [72]. The growth of plantlets under photoautotrophic conditions reduces loss of plantlets, increase water use efficiency and photosynthesis resulting in increasing plantlet survival after acclimatization [73], also potato plantlets grown in photoautotrophic conditions were healthier than the conventionally grown plantlets in terms of number and size of leaves, observed after 4 weeks of acclimatization [74].

7. ALTERNATIVES TO OTHER INGREDIENTS

Many other ingredients can also be replaced by low cost options, commercial grade chemicals of lower purity than the analytical grades can be used for commercial micropropagation unless deleterious effects are observed. A high degree of purity is justified only in the case of basic studies in tissue culture. In general commercial micropropagation, the quality will not be much affected ordinarily by purity of these chemicals. Growth regulators (hormones) are the most expensive out of all the chemicals; however, they are needed in very small amounts in the medium, thus having a little effect on the medium cost [5]. Sugar cane molasses can provide many of the nutrients, namely, sugar, vitamins and inorganic metal ions required for sugarcane callus induction and shoot formation [75].

7.1 Tomato *in vitro* Culture without Growth Regulators

It was reported that Tomato cut seeds with proximal hypocotyl portion cultured on a medium with Murashige and Skoog salts, Gelrite 2 g.L-1, Mio-Inositol 100 mg.L-1, Thiamine 4 mg.L-1 , 3% commercial sucrose, without growth regulators gave more than 60% adventitious shoot formation after 2-3 weeks of culturing [76].

7.2 Cowpea *in vitro* Culture without Growth Regulators

The effects of tomato juice as hormonal supplements in the embryo (*in vitro*) culture of cowpea variety (Ife brown and TVU 943) was studied [77]. From the study it was concluded that use of 15% tomato juice as hormonal supplement in *in vitro* tissue culture is best for root length, shoot number, root number and leaf number while a decrease in plant height and shoot length was noticed when compared with the control (MS medium only) experiment.

8. LOW COST WATER ALTERNATIVES

Water is the main component of all plant tissue culture media and distilled or doubled distilled and de-ionized water is most commonly used. Distilled water produced through electrical distillation is expensive and adds to cost of tissue culture. Tap water (free from heavy metals and contaminants) can be substituted for distilled water to lower the cost of the medium [17,78]. Tap water after autoclaving can be used in small facilities rather than distilled water. RO water can be used for stock solutions and hormone preparations and distilled water for media preparation to reduce the cost most effectively. It also is used for washing plants prior to sterilization and also for the purpose of added sterilants for cleaning. Table bottled water from the supermarket can also be used as low cost alternative. However, its mineral composition should be taken into account as it may affect pH and nutrient uptake (H.J. Jacobsen, University of Hannover, Personal communication). In rural areas, rainwater can be collected in clean glass jars and used for tissue culture. In Bangladesh, the change over of water distillation from electrical to gas operated unit reduced the cost from US$260 to $5/month for producing 50-60 liter water per day (A. Razzaque, BRAC Biotech, Personal communication).

Potato *in vitro* culture was done using 9 different types of water viz., rain, natural, tap, aqua-guard, single distilled, double distilled, Type-I (Reverse osmosis), Type-II (Electronically de-ionized) and ultra-pure water [55]. From the observations it was concluded that clean tap water can be used for media preparation which will reduce investment on costly apparatus as well as electricity.

9. USE OF NATURAL LIGHT AS LOW COST LIGHTING ALTERNATIVE

Artificial lighting of cultures within the growth rooms is one among the foremost expensive and inefficient methods in tissue culture technology, the lighting equipments and their operation and maintenance add to high costs. Moreover, artificial lighting generates heat that has to be dissipated by cooling and air conditioning further adding to the electrical load. Although special fluorescent tubes are used to compensate for the red and far-red part of natural daylight, artificial light quality does not match that of natural light under which the plants are ultimately grown. Also, the cool fluorescent lights used for illumination provide minimal energy required for photosynthesis, as a result, in vitro plants adapt to low-light intensity, and have a reduced growth rate. Under artificial light of low intensity, plants have low food reserves, and a poorly developed root system and when they are transferred to soil, the *in vitro* formed roots have to adjust to soil solutes of varying pH. The usual response of the *in vitro* formed roots is that they stop functioning in soil and new roots are formed, which take over the function of the original roots and if a new roots does not emerge, the plant dies. One method to circumvent these negative effects is to culture the plants under natural light, during their last phase in liquid medium, based on half- or quarter-strength MS salts without sugar and vitamins, under either aseptic or non-aseptic conditions. If roots or root initials are not formed, the medium can be either supplemented with auxins (IAA, IBA), or shoots dipped in a solution of rooting hormones. This procedure provides much stronger and healthier plants with a high survival rate. It has been tested that plants hardened under natural light are hardy and withstand better in the field after transplantation [9]. Natural light has been successfully used in this manner in “Bio-factories” in Cuba, based on the conversion of village houses into tissue culture laboratories [79].

The conventional micropropagation conditions of maintaining the *in vitro* plants in a controlled room were replaced with a room whose roof was made of corrugated plastic sheets that allow partial passage of natural light in potato cultivar ‘Diamant’ [80]. From this research no differences were found between yield production of micropropagated plants grown under control and non-controlled conditions. The effect of natural sunlight on potato micropropagation was studied and the results were compared with the cultures produced through artificial light [81]. It was found that all the growth factors gave better result in sunlight treatment than those of artificial one except average number of nodes and leaves. An experiment was conducted with nine replications under *in vitro* conditions and four under greenhouse conditions for two varieties (Agria and Savalan) [82]. The results indicated that in variety “Savalan” the plant height is almost similar in both light conditions while a higher plant height was found in florescent light in “Agria”. Root length, stem diameter, leaf area, number of nodes per plantlet, number of branches per plantlet was higher in natural light in both cultivars.

10. LOW COST CONTAINERS

Glasswares normally used in plant tissue culture (test tubes, conical flasks, glass and plastic petri dishes) are expensive and a wide variety of containers have been tested at different stages of micropropagation. Pre-sterilized disposable-plastic petri dishes, glass bottles and baby food jars with polypropylene caps are cheaper and have been tested and found as an economic and low cost option. Autoclavable transparent plastic containers and containers made of polypropylene, polycarbonate and polystyrene are used in many countries. Gamma ray sterilized non-autoclavable food containers, polystyrene sandwich boxes, plastic bags, PVC pots and jars are being used for large scale micropropagation [5]. Culture vessel like ‘StarPac’ disposable bag, ‘Watson Modules (plastic type container) are being used at different stages (hardening, multiplication, soil growing) of plant growth [83,84,85]. Juice, Jam and jelly bottles and even old whisky bottles are used in Bangladesh, India, Thailand, Singapore, Indonesia, Malaysia. Vessel closures and lids play important role for growth of in vitro plants, in normal practice non-absorbent cotton plugs, polyurethane foam plugs, plastic plugs, aluminium foil, stainless steel caps, polypropylene caps, PVC film, polythene film,

silicon rubber etc. are used. For large scale production such caps have been replaced by autoclavable screw caps made of stainless steel or polypropylene [86,87,88,89,90].

11. CONCLUSION

The potential of plant tissue culture in increasing agricultural production and generating rural employment is well recognized by both investors and policy makers in developing countries. However, in many developing countries, the establishment cost of facilities and unit production cost of micropropagated plants is high, and often the return on investment is not in proportion to the potential economic advantages of the technology. These problems can be addressed by standardizing agronomic practices more precisely (precision agriculture) and by achieving maximum net profits from the crops or by decreasing the unit cost of production or both. Using low cost alternatives for agar, sucrose, glass wares, light, pressure cooker instead of autoclave, tap water instead for the distilled water and other low cost alternatives we can greatly reduce the cost of tissue culture. Low cost tissue culture technology can enable us to exploit the potential of tissue culture for sustainable production in developing countries such as India and help bring tissue culture from the labs to the farms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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