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Low Cost Tissue Culture Technology for the Regeneration of Some Economically Important Plants for Developing Countries

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Abstract

Plant tissue culture, an ecofriendly technology includes micropropagation which leads to mass propagation of true to type, high quality planting material of ornamentals, medicinal crops plantation crops, fruit and forest trees etc within a limited period . It has provided challenging opportunities in global trading of tissue culture saplings as well as cut flowers for export as well as for domestic use in developing countries like India. This area has created new avenues for entreprenureurs. But the main bottleneck is the high cost of tissue culture planting materials compared to the conventionally propagated saplings. It is a capital-intensive industry, and in some cases the unit cost per plant becomes unaffordable. Hence, it is necessary to adopt strategies to reduce production cost and lower the cost per plant. This paper deals with various low cost tissue culture techniques which can be adopted by small scale entreprenures in Indian conditions Bioreactors provide more precise control of the plant growth gaseous exchange, illumination, medium agitation, temperature and pH than the conventional culture vessels. However, to be cost-effective, use of bioreactors requires indexed plant cultures, and attention to aseptic procedures during handling of plant material otherwise culture contamination leads to massive economic loss. Plants hardened under natural light are sturdy, and withstand transplantation better in the field. Careful planning of a facility can make large savings both in the construction costs and day-to day operations in the facility. The primary application of micropropagation has been to produce elite planting material irrespective of season or crop, which in turn leads to increased productivity in agriculture as well as better economy to developing nations like India.

Keywords: Plant tissue culture, micropropagation, micropropagules, low cost tissue culture techniques

Introduction

Plant tissue culture refers to growing and multiplication of cells, tissues and organs of plants on defined solid or liquid media under aseptic and controlled environment. Micropropagation allows rapid production of high quality, disease-free and true to type planting materials within a short duration in limited space. The micropropagation leading to mass propagation of high quality planting material

of ornamentals, and forest and fruit trees has created new and challenging opportunities in global trading for producers, farmers, and nursery owners (FAO,2003a). It also leads to improvement of our economy particularly leads to rural employment. Tissue culturing has become a routine method for propagating plants in high technology laboratories (de Fossard,1976). The cost of production using conventional tissue culture is, however, high for most of the countries in the developing countries. Although conventional Plant Tissue Culture (PTC) has been applied for decades, the high cost of tissue production is a drawback for laboratories with limited resources, especially in the developing countries. High production cost has been an impediment to tissue culture adoption especially in developing countries such as India and sub-Saharan Africa. In fact, the cost of the primary production precludes the adoption of the technology for large scale micropropagation.

Role of low cost options in plant tissue culture

Plant tissue culture is primarily based on rapid multiplication of tiny stem cuttings, axillary buds, and to a limited extent of somatic embryos, cell clumps in suspension cultures and bioreactors. The plants can be multiplied anywhere under controlled environmental conditions throughout the year irrespective of the season and weather (Etienne et.al, 1999). The cultured cells and tissue can take several pathways. The pathways that lead to the production of true-to-type plants in large numbers are the preferred ones for commercial multiplication. However, tissue culture technology is more expensive than the conventional method of plant propagation because of the initial capital input followed by maintenance of the high- tech equipment involved and the controlled conditions required in addition to the skilled labour. Therefore, it is a capital-intensive industry, and in some cases the unit cost per plant becomes unaffordable. Hence, it is necessary to adopt strategies to reduce production cost and lower the cost per plant. The process of plant tissue culture is usually divided into several stages.

1. Identification and maintenance of elite mother plants
2. Their pre-propagation,
3. initiation of explants,
4. subculture of explants for proliferation,
5. shooting and rooting,
6. hardening followed by
7. Lab to land transfer

These stages are universally applicable in large-scale multiplication or mass propagation of plants. The delivery of hardened small plants / plantlets of tissue culture to growers and market also requires extra care. Low-cost tissue culture technology is the adoption of practices and use of equipment to reduce the unit cost of plantlets and overall plant production (FAO,1993). Low cost options should lower the cost of production without compromising the quality of the micropropagules and plants. In low cost technology cost reduction is achieved by improving process efficiency and better utilization of resources. Low-cost tissue-culture technology is high priority in agriculture, horticulture, forestry, and floriculture of many developing countries for the production of suitably priced high quality planting material.

A number of low-cost alternatives can be used to simplify various operations and reduce the costs in a tissue culture facility. The physical components of a typical plant tissue culture facility include equipment and buildings with preparation room, transfer room, culture or growth room, hardening and weaning area, soil-growing area (greenhouses, plastic tunnels), packaging and shipping area, and related facilities such as an office, and a store for chemicals, containers and supplies. The size of the physical components of a tissue culture facility will vary according to its functional needs, *i.e.* the volume of production.

Careful planning of a facility can make large savings both in the construction costs and day-to day operations in the facility. It is recommended that an existing facility should be visited to view the layout and operational needs before starting a new facility. Proper choice of media and containers can reduce the cost of micropropagation (FAO,2000b). The composition of culture media used for proliferation has a tremendous influence on production costs. The type of culture vessel influences the efficiency of transfer during subculture and production of propagules per unit area. The replacement of expensive imported vessels with reusable glass jars and lids, alternatives to gelling agents, use of household sucrose, and some medium components can reduce costs of production. Bulk making of media and storage as deep frozen stocks also reduces labour costs.

Artificial lighting of cultures in the growth rooms is one of the most expensive and inefficient methods in tissue culture technology. Changing the method of illumination from artificial to natural light is a decisive low cost option in tissue culture. This reduces electricity and capital costs

and also improves the plant quality. Maintaining in vitro cultures at a regulated temperature with air conditioners adds to the cost but does not contribute to specific plant quality. Many in vitro growing plants can tolerate wide fluctuations in temperature, and adapt better to field conditions than those grown under even temperature.

Plants can also be hardened in open shade. Plants hardened under natural light are sturdy, and withstand transplantation better in the field. Production of plants based on tissue culture technology and their subsequent growing is a labor intensive system. Even in developing countries, where labor is relatively less costly, hiring expertise from established R&D laboratories reduces overhead costs.

Bioreactor-based propagation of plants can increase rate of multiplication and growth of cultures and reduce space, energy and labor requirements in commercial micropropagation. They can therefore be attractive to developing countries as regards new or expanding plant culture facilities, in combination with a conventional laboratory. Bioreactors provide more precise control of the plant growth gaseous exchange, illumination, medium agitation, temperature and pH than the conventional culture vessels (Adelberg and Simpson,2002). However, to be cost-effective, use of bioreactors requires indexed plant cultures, and attention to aseptic procedures during handling of plant material otherwise culture contamination leads to massive economic loss.

In plant tissue culture the health status of the donor mother plant and of the daughter plants multiplied from it are among the most critical factors, which determine the success of a tissue culture operation. Plants not originating from pathogen-tested material must be screened for the presence of viruses. The process termed indexing of the mother plants for freedom from viral, bacterial, and fungal diseases is a normal procedure in large-scale plant propagation through tissue culture. Batches of tissue cultured plants should be tested for freedom from diseases either in-house or by other laboratories. Laboratories, which do not have in-house facilities to carry out plant indexing, should either procure their indexed stock plants from organizations such as Departments of Agriculture, agricultural universities or privately owned certified germplasm repositories that routinely produce such plant material or can get stock cultures indexed in certified laboratories For virus and pathogen detection ELISA has been the most effective method. Polymerase chain reaction (PCR) and nucleic acid hybridization are more sensitive than ELISA, and can detect pathogens in extremely low amounts. Quality control is essential to assure production of high quality plants and to have end-users confidence. Quality standards require the establishment of suitable tests to maintain quality control. The choice of explant source, freedom of the donor plant from viruses, disease causing fungi, bacteria, viroids, phytoplasmas, vigour and conformity of the variety, and elimination of somaclones are critical for maintaining plant quality (Ahloowalia, 2000). Variety identification by proper labeling at all stages is essential to ensure varietal identity.

Tissue-cultured propagules are produced under a controlled environment. Such plantlets have small juvenile leaves with reduced photosynthetic capacity, and malfunctioning stomata. Priming for rooting, shooting, and improved photosynthesis can be achieved with growth regulators and adjustment to the growing conditions that affect the post-transplanting performance of the propagules. Vented closures with microbial filters facilitate gas exchange, reduce ethylene build-up that stunts plant growth, reduces leaf-size, and causes leaf drop in tissue culture containers. Plantlets produced under photo-autotrophic culture systems on media with or without sucrose but CO_2 enrichment, increased light intensity, good gas exchange and reduced humidity are more vigorous, have larger root-systems, and are less susceptible to microbial contamination. Plants adapted gradually to the ex-vitro environment have improved survival upon transfer to soil. Plants in their natural environment are colonized with many bacteria, fungi, and mycorrhizae. *In-vitro* or *ex-vitro* biopriming of micropropagated plants with such organisms improves plant performance under stress environments, and consequently enhances yield.

Low cost options can generally be incorporated into the design of the building, laboratories, working areas, layout of equipment, lighting, heating and production planning to provide smooth and efficient operations. It is important to select several plants that provide options for production around the year to allow cash flow and optimal use of equipment and facilities. It is essential to maintain sufficient mother cultures, and limit the number of subcultures to avoid variation, and plan production and shipment according to the customer's demand. The price of the tissue culture derived plants, tubers, bulbs, and cuttings must be competitive with those obtained from conventional propagation. Many tissue-cultured plants are too expensive for direct field planting. In such cases, the cost can be reduced by one or more conventional propagations of the in vitro plants. The uniformity and consistency in field

performance of tissue-cultured plants is important to build confidence of the farmers to integrate such plants in the production systems.

Adoption of Low-Cost Options

Low cost options should lower the cost of production without compromising the quality of the micropropagules and plants. 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. The foremost requirement of micropropagation is the aseptic culture and multiplication of plant material (Gross and Levin,1999) .

Importance of Low Cost Technology

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. The technology is particularly relevant to the propagation of ornamental plants. Despite high costs of production, trading of ornamental plants has thrived because they command high unit value. However, the market is limited. Over a period of time, many new tissue-culture companies in several developing countries have entered to compete in the limited market. The inevitable result has been the reduction of net margins below viable limits (Table 1).

Low Cost Energy Options

Use of natural light

Artificial lighting of cultures in the growth rooms is one of the most expensive and inefficient methods in tissue culture technology. The lights, chokes, fixtures, timer controls, equipment to handle high electrical load, and their operation and maintenance add to high costs. Moreover, artificial lighting generates heat that has to be dissipated by cooling and airconditioning 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.

Plants can adapt to a wide range of conditions by changing their metabolism and structures. They develop structural and anatomical features in leaves and stems, e.g. cuticle and wax on leaves, thickness of the leaf, fewer and closed

Table 1: Substitution of plant tissue culturing equipment and facilities

Sl. No.	Equipment	Cost in KES (1 US$= 80 KES)	Substitute low cost equipment	Cost in KES (1 US$= 80 and facility KES)	% cost saving
1.	Autoclave	70000.00	Pressure cooker	10000.00	87.8
2.	Culturebottles	450.00	Jam jar bottles	15.00	96.7
3.	Micropipette	250.00	Insulin syringes	10.00	96.0
4.	Measuringcylinders	250.00	Vet syringes	10.00	96.0
5.	Petri dish	7.00	Office waste papers	1.00	85.7
6.	Aluminium foil	145.00	Office waste papers	1.00	99.3
7.	Subtotal	71102.00		10037.00	85.9
8.	Facility				
9.	Greenhouse	800000.00	Shade net	48000.00	94.0
10.	Grand total	871102.00		58037.00	93.3

Source: Gitonga *et al.* African Crop Science Journal, Vol. 18, No. 4, pp. 243 – 251, 2010 Tissue culture materials for initiation and multiplication of banana

stomata, and thickening of epidermal cells of the stems, that allow survival in the harsh environment. However, once they adapt to a set of conditions, re-adaptation to new conditions is rather slow or difficult. Plant tissues formed and adapted to low light conditions are usually fragile and may become vitrified, leading to poor survival under field conditions. This can be a major disadvantage in the plant hardening process, and later establishment in the field.

Plants have low reserves, and a poor root system under artificial light of low intensity. On transfer 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. If new roots do not emerge, the plant dies. One method to circumvent these negative effects is to culture the *in vitro* 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.

Cost effective techniques of plant tissue culture in Indian conditions

Tissue culture is one of the boon to the agro based industry throughout the world particularly India . Plant tissue culture techniques has been used for the production of disease free plants in large scale also it has practiced for the *in vitro* and aseptic growth of the plant part in the nutrient medium for producing plantlets. The main objective of tissue culture is to produce high yielding varieties of crops without compromising the quality of the plantlets. Tissue culture (PTC) plays an important role for culturing both cash crops and ornamental plants. Low cost technology means the adoption of technological practices and also the making use of the equipment to reduce the propagating plantlet cost as well as their production.

Components of tissue culture for cost reduction

There are three important tissue culturing components which involves in the reduction of the cost

1. Chemicals
2. Equipments
3. Structures

1. Chemicals

Minerals nutrients, plant growth hormones, vitamins. The chemicals which here focuses on the macronutrients, micronutrients, vitamins, amino acids or nitrogen supplements, sources of carbon, growth regulators and solidifying agents. International Association for Plant Physiology encompasses that, the concentrations of elements greater than 0.5 mM. are defined as macroelements and those required in concentrations less than 0.5 mM as microelements (de Fossard,1976).

Macronutrients

Plant tissue culture media contains macroelements: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) for better growth. At least Culture media should contain 25-60 mM of inorganic nitrogen for good plant growth. Potassium (K) is required for proper cell growth of various plant species. Most of the media contains potassium (K) in the form of nitrate chloride salts with a concentration ranging from 20 – 30mM. The optimum concentrations of P, Mg, S and Ca range from 1-3 mM if other requirements for cell growth are provided (Torres,1989).

Micronutrients

The important micronutrients for plant tissue growth include iron (Fe), manganese (Mn), zinc (Zn), boron (B), copper (Cu) and molybdenum (Mo). Iron is usually the most critical of all the micronutrients. The element is used as either citrate or tartarate salts in culture media, however, there exist some problems with these compounds for their difficulty to dissolve and precipitate after media preparation. (Murashige and Skoog 1962).

Carbon and energy sources

In tissue culture media, sucrose which is frequently used as carbon source at a concentration of 2-5%, other carbohydrates are also used. In order to reduce the tissue culture cost other sugar supplements such as sugar cane molasses, banana extract and coconut water were added to the basal media. The substrates in addition to sugars, they are sources of vitamins and inorganic ions required growth (Dhamankar,1992; Zahed ,2000). (Vasil & Thorpe, 1998).

Vitamins and myo-inositol

Vitamins should be added in the culture media only when the concentration of thiamine is low (Murashige, 1974).

Myo inositol (carbohydrate) is added in small quantities to stimulate cell growth of most plant cell (Vasil & Thorpe, 1998).

1. Equipments

Culture containers, laminar flow, autoclave, instruments used for micropropagation, pH meter etc

Limitations of conventional tissue culture

- High cost of equipment
- High cost of nutrient media and sterilizing agents
- High cost of facilities
- shortage of trained personnel
- Lack of systems for marketing/ delivering tissue culture products

The usage of a sterile hood was found satisfacton, for carrying nut all the culture manipulations involved in this studv. However. compared to a laminar flow bench the hoods have confined space and restrict the hand movement

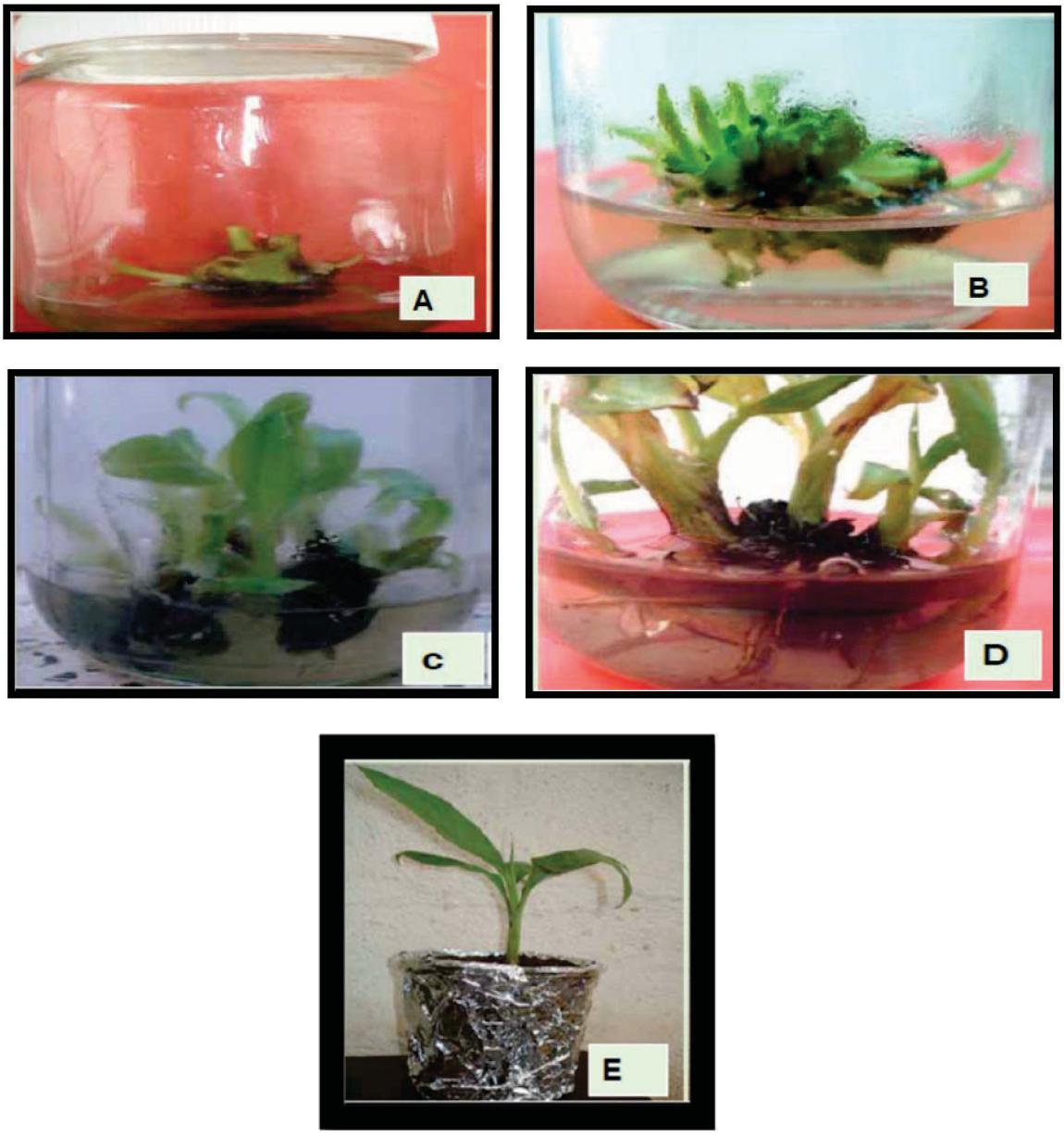

Fig.1: A-E Comparison between the low cost medium and the conventional medium sources

of the operator. The use of PP bags for culture is particularly rendered more difficult. It requires repeated practice to reduce contamination rates in the bags.

Low Cost Tissue Culture Technolgy of Cash Crops

1. Sweet potato (*Ipomoea batatas* (L.)

In africa Sweet potato (*Ipomoea batatas* (L.) Lam) is the second most important root crop after cassava. (Kwame Okinyi Ogero *et.al*, 2012) has focused to reduce the cost of sweet potato tissue culture nutrients by using affordable alternative nutrient sources (Kodym *et.al.*, 2012). He developed a new medium with conventional sources of Murashige and Skoog (MS) salts were substituted with Easygro® vegetative fertilizer containing both macro and micronutrients. Easygro® vegetative fertilizer, a locally available foliar feed was used as the alternative source of MS nutrients. Two grams of the fertilizer which contains both macro and micronutrients were used to make one litre of medium. This was supplemented with 30 g/L of table sugar and 9 g/L of agar. The use of Easygro vegetative fertilizer as the alternative source of MS nutrients reduced the cost of the nutrient medium by 96.2% while the use of table sugar led to 97.1% savings in regard to the source of carbon A total cost reduction of 96.9% was realized as shown in Table 1. (Kwame Okinyi Ogero *et.al*, 2012).

2. Low cost tissue culture technology for banana plants

Low cost banana tissue culture was carried by substituting the following ingredients such as normal tap water, table sugar, agar is replaced by isabgol in MS medium. In the case of root initiating MS medium some other ingredients were substituted such as saw dust, groundnut husk, rice husk, coir pith and coconut fibre were used instead of agar. The growth seems to similar compared with original medium (Das & Gupta, 2009; Kodym and Zapata,1999 ; Kodym and Zapata,2001)

3. Low cost medium for sugarcane tissue culture

Norhayati et.al 2011 used four kinds of commercial starch or flour as alternative gelling agents and coconut water as an organic additive in the culture medium has reduced the cost of the medium and there is no significant growth difference compared to the original gelling agents.

4. Cheaper Alternatives to MS Media for *in vitro* Culture of Potato

In low cost media, tapioca was used as substitute of agar and replacing sucrose with sugar cane, because of low cost and easy availability (Gitonga *et.al*, 2011). Calcium ammonium nitrate, Single super phosphate Muriate of potash and sugar cane were used as low cost media in place of MS salts. The plants produced using LC media was consistently better for shoot and proliferation. It is concluded that through reduction of the cost on the techniques, the cost of the product also be reduced and farmers get benefited using low cost, disease free and clonal planting material with high production and also saving land resources (Badoni and Chauhan, 2011).

Conclusion

It has been stressed time and again that in the long-term agriculture and forestry need to be sustainable, use little or no crop-protection chemicals, have low energy inputs and yet maintain high yields, while producing high quality material. Biotechnology-assisted plant breeding is an essential step to achieve these goals. Plant tissue culture techniques have a vast potential to produce plants of superior quality, but this potential has been not been fully exploited in the developing countries. During *in vitro* growth, plants can also be primed for optimal performance after transfer to soil. In most cases, tissue-cultured plants out-perform those propagated conventionally. Thus *in vitro* culture has a unique role in sustainable and competitive agriculture and forestry, and has been successfully applied in plant breeding, and for the rapid introduction of improved plants.

Bringing new improved varieties to market can take several years if the multiplication rate is slow. For example, it may take a lily breeder 15-20 years to produce sufficient numbers of bulbs of a newly bred cultivar before it can be marketed. *In vitro* propagation can considerably speed up this process. Plant tissue culture has also become an integral part of plant breeding. For example, the development of pest- and disease-resistant plants through biotechnology depends on a tissue culture based genetic transformation. The improved resistance to diseases and pests enables growers to reduce or eliminate the application of chemicals. Plastic tunnels (polytunnel) of low-cost with bio-fertilization can also be used for hardening, especially in cooler climates. A rectangular pit of desired and manageable size is dug on a site free of water logging. A frame of bamboo or any other available material is made above the pit with a gentle slope to one side. The frame is then covered with a clear polythene sheet, and tied using nylon ropes. The sheet is sealed with mud on three sides, leaving one side free. The

temperature and humidity inside these structures is generally higher than the outside, and protects the plants from frost under cold climate . As and when required the polythene sheet can be partly opened to allow air circulation and sunlight. The added advantage of polypits is the carbon dioxide fertilization effect and reduced need for watering. Another low cost hardening alternative is to hang the plastic bags under tree shade which the farmers employed in hardening can adopt. Thus micropropagation has proved especially useful in producing high quality, disease-free planting material for a wide range of crops. Tissue cultured based industry also generates much-needed rural employment, particularly for women.

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References

Adelberg, J.W. and Simpson, E.P. 2002. Intermittent Immersion Vessel Apparatus and Process for Plant Propagation. Internl. S/N: PCT/US01/06586.

Ahloowalia, B.S. 2000. Stability of micropropagated plants and mini-tubers of potato. In: Methods and Markers for Quality Assurance in Micropropagation. Cassells, A.C., Doyal, B.M. and Curry, R.F. (Eds.) Proc. Internl. Symp. Cork, Ireland Pp.163-164.

Anonymous. 2001. Solatube, the miracle skylight. http:// www.solaglobal.com

Anonymous. 2002. Indian tissue culture industry, Part-I. Dalal Consultants and Engineers Ltd. Floriculture Today (June), India

Anoop Badoni and J.S. Chauhan. Some of Cheaper Alternatives to Ms Media for in vitro Culture of Potato. *Libyan Agriculture Research Center Journal International*, **2**(4): 161-167, 2011

Barathi, N. 2002. Plant TC list serv. www.agro.agri.umn.edu/plant-tc/ listserv/

Das A, and Gupta S N. 2009. Use of low cost resources for banana micropropagation. *Indian Journal of Horticulture*, : **66:**3-15

Fossard, de R. Tissue culture for plant propagation. Armidale: University of New England; 1976.

Dhamankar V.S.1992 Molasses, a source of nutrients for *in vitro* sugar cane culture. *Sugar Can*e 1992; **4:** 14- 15.

Etienne, E., Teisson, C., Alavard, D., Lartaud, M., Berthouly, M., Georget, F., Escalona, M. and Lorenzo, J.C. 1999. Temporary immersion for plant tissue culture. In: Plant Biotechnology and In Vitro Biology in the 21stCentury. (Eds. Altman, A., Ziv, M. and Izhar, S.). Kluwer Acad. Pub. Dordrecht. 633-636.

FAO, 1993. Agricultural Biotechnology in the Developing World. FAO Research and Technology

FAO, 2000a. How appropriate are currently available biotechnologies in the crop sector for food production and agriculture in developing countries. Electronic forum on biotechnology in food and agriculture: Conference 1. Food and Agriculture Organization of the United Nations, Rome, Italy

FAO, 2000b. How appropriate are currently available biotechnologies for the forestry sector in developing countries. Food and Agriculture Organization of the United Nations, Rome, Italy http://www.fao.org/biotech/Conf2.htmFood and Agriculture Organization of the United Nations, Rome, Italy

Gitonga, N.M, O. Ombori, K.S.D. Murithi and M. Ngugi Norhayati Daud, Rosna Mat Taha, Nor Nafizah Mohd Noor and Hasimah Alimon. Provision of low cost media options for in vitro culture of Celosia sp. African Journal of Biotechnology Vol. 10(80), pp. 18349-18355, 14 December, 2011

Gross, A. and Levin, R. 1999. Design considerations for a mechanised micropropagation laboratory. In: Plant Biotechnology and In Vitro Biology in the 21stCentury. A. Altman, M. Ziv and S. Izhar (Eds.) Kluwer Acad. Pub. Dordrecht. Pp 633- 636.

Kodym, A. and Zapata-Arias F.J. 2001. Low-cost alternatives for the micropropagation of banana. *Plant Cell Tiss. Org. Cult*. **66:** 67-71.

Kodym, A. and Zapata-Arias, F. J. 1999. Natural light as an alternative light source for the in vitro culture of banana (*Musa acuminata* cv. 'Grande Naine'). *Plant Cell Tiss. Org. Cult.* **55:** 141-145.

Kodym, A., and Zapata-Arias, F.J. 2001. Low-cost alternatives for the micropropagation of banana. Plant Cell Tiss. Org. Cult. 66: 67-71.

Kodym, A., Hollenthoner, S. and Zapata-Arias, F.J. 2001. Cost reduction in the Kwame Okinyi Ogero, Gitonga Nkanata Mburugu , Maina Mwangi, Michael Mugambi Ngugi and Omwoyo Ombori. Low Cost Tissue Culture Technology in the Regeneration of Sweet Potato (Ipomoea batatas (L) Lam). *Research Journal of Biology* (2012), **2**(2):51-58

Kwame Okinyi Ogero, Gitonga Nkanata Mburugu , Maina Mwangi , Michael Mugambi Ngugi and Omwoyo Ombori. Low Cost Tissue Culture Technology in the Regeneration of Sweet Potato (*Ipomoea batatas* (L) Lam) *K.O. Ogero et al / Research Journal of Biology*, (2012), **2**(2):51-58 ISSN 2049-1727

Leifert, C. 2000. Quality Assurance Systems for Plant Cell and Tissue Culture: The problem of Latent Persistance of Bacterial Pathogens and Agrobacterium Transformed vectorsystems. In. Proc. Intl. Symp. Methods and Markers For Quality Assurance in Micropropagation. A.C. Cassells et al. (Eds.) Acta. Hort. Pp. 530.

Murashige T, Skoog F. A. 1962. revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant*.**15**: 473-479.

Murashige T. Plant propagation through tissue cultures. *Ann. Rev. Plant Physiol*. (1974); **25:**135-166.

Prakash, J. and Peirik, R.L.M. 1991. Horticulture - New Technologies and Applications. Kluwer Acad. Pub. Dordrecht. Pp.414.

Savangikar, V.A., Savangikar, C., Daga, R.S. and Pathak, S. 2002. Reduction in cost in micropropagation: achievements and further prospects. 1st Internl. Symp. 'Liquid System for *In*

Vitro Mass Propagation of Plants', Agric. Univ. Norway, Ås, Norway 29 May - 2 June, 2002

Torres K.C., editor. Tissue culture techniques for horticultural crops. New York, London: Chapman and Hall; 1989.

Vasil I.K, Thorpe T.A, editor. Plant cell and tissue culture. Dordrecht: Kluwer Acad. Publ.; 1998

Zahed M.A.2000. 2000 Studies on morphogenesis of three elite species of orchids. Thesis MSc, Univ. Dhaka, Bangladesh.

