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Cost effective tissue culture technology for clonal propagation of pineapple in Ethiopia: A review article

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Abstract
Clonal propagation is a plant tissue culture technique used for producing plantlets and implies the culture of aseptic small sections of tissues and organs in vessels with defined culture medium and under controlled environmental conditions and has become an increasingly important tool for both science and commercial applications. Low-cost tissue culture technology is the adoption of practices and use of equipment to reduce the unit cost of micropropagule and plant production. Low cost options should be lower the cost of production 25% from electric city cost, 65% from agar, 82% from table sugar, rain water and locally prepared soil media had been cost effective by increasing the quality of the clonal propagation, time and space saving and plants production. In the low cost technology cost reduction is achieved by improving process efficiency and better utilization of resources. Low-cost tissue-culture technologies have been stay a high priority in agriculture, horticulture, forestry, and floriculture of many developing countries for the production of suitably priced high quality planting material. Tissue cultured based industries have also generates much-needed rural employment, particularly for genders participants.

Keywords: low cost**,** *Ananas comosus,* tissue culture and clonal propagation

Introduction

Pineapple (*Ananas comosus* (L.) Merr.) is economically important edible plant of the family Bromeliaceae that is cultivated in most tropical and subtropical countries ranking third in world production among tropical fruits, after banana and citrus. The proliferation of pineapple plants for commercial-scale farming conventionally uses suckers, crowns, hapas, or slips from field-grown plants as planting substantial. However, conventional pineapple propagation through sucker, crown, slip and hapa unable to gratify the demand representation by farmers and commercial plantations for disease free and uniform plantlets. Micropropagation is a plant tissue culture technique used for producing plantlets and implies the culture of aseptic small sections of tissues and organs in vessels with defined culture medium and under controlled environmental conditions and has become an increasingly important tool for both science and commercial applications. Micropropagation technique has been successfully active for *in vitro* mass propagation, diseases free and true to type pineapple plantlets in a small unit area. In this regard, Jimma Agricultural Research Center (JARC), Plant Biotechnology Laboratory has been distributed more than 1.2 million plantlets of pineapple cv. Smooth Cayenne and MD2 to end users. Micropropagation allows rapid production of high quality, disease-free and uniformity of planting materials within a short period of time in a limited space. The micropropagation leading to mass propagation of high quality planting material of seedlings has created new challenging and opportunities in global trading for producers, farmers, and nursery owners (FAO, 2003a). When this approach was applied, an increase of 40- to 85-fold in shoot number over a 13-months period was reported for pineapple cv. Smooth Cayenne (Kiss *et al*. 1995) [12]. These results agree with data reported by (Teixeira *et al*., 2005b, c, d, e) [16], authorizing their conclusions that nutrient media for plant tissue culture can be sterilized without autoclaving by adopting a new protocol in which all the instruments used for preparing and packing the medium are sterilized with sodium hypochlorite, and a low concentration of the same sterilizing agents were added directly to the MS media. Hence, this cost also indicated by (Biruk A., *et al*.,2012) [2] Enset flour, 'Bulla', as an alternative gelling agent for pineapple *in vitro* micropropagation and cost reduction sustained from using expensive gelling agents. The highest costs of media come from the use of analytical tissue culture grade sucrose (Demo *et al*., 2008) [7]. Prakash, Hoque, and Brinks (2004) [13] reported that, the main component of all tissue culture media was normally considered to be free from ions and contamination or double-distilled water. Recently, the use of high cost energy sources have been replaced by cheap and locally accessible carbohydrate sources such as table sugar revealed promising responses. Therefore, this review was commenced to observe the interaction effects of the different chemical sterilization, gelling agents, type of water, carbon sources and acclimatization during clonal propagation of tissue culture technology by adopting low cost substituent in MS media to make economical affordable.

Cost effective techniques of plant tissue culture in Ethiopia

Tissue culture is one of the advantages to the agro based industry throughout the world particularly Ethiopia. Plant tissue culture techniques has been used for the production of disease free plantlets in large scale also it has practiced for the *in vitro* and aseptic growth of the plant part in the prepared nutrient medium for the production of many seedlings. The main objective of tissue culture is to produce high yielding varieties of crops without compromising the

quality of the plantlets. Plant Tissue culture plays an important role for culturing both cash crops and ornamental plants. Low cost technology means the adoption of technological practices and also making use of equipment's to reduce the cost micro propagating as well as advance their production system.

Chemical sterilization

The alternative technique for sterilization of culture medium to replace autoclaving was carried out for reducing the cost of electric city. For sterilization of culture medium without autoclaving was provided to 50 ml Murashige and Skoog (MS) medium before medium was solidified and kept for two weeks before evaluating sterile conditions. Treated media, supplemented with sodium hypochlorite (NaOCl), were compared to the control medium, autoclaved at 121 degree Celsius for 20 min. *In vitro* sterile conditions were found 20–100% from these treated media compared to 95% sterile condition from autoclaved medium. Treated media obtained 100% sterile conditions at 0.0025% (0.5ml/L) active chlorine were chosen limit concentration of 5 % (v/v) sodium hypochlorite provide for culturing of shoot, root and fresh biomass (Berihu M. 2018). Therefore, the development of this technique, using chemical sterilization of sodium hypochlorite corresponding formula was NaOCl for eradicating microorganisms causing agents of contamination, to replace the autoclaving, time consuming and 25% cost of electric city for establishing sterile culture medium and inadequate electric power were the best opportunity for *in vitro* culture.

Gelling agents

The growth of cultures and production of shoots or roots is strongly influenced by the physical consistency of the culture medium. Gelling agents are usually added to the culture medium to increase its viscosity as a result of which plant tissues and organs remain above the surface of the nutrient medium. Many gelling agents are used for plant culture media, e.g., agar, 'Agarose', and 'Gellan gum', and are marketed under trade names such as 'Phytagel, Gelrite' (Sigma Co., Merck & Co. Inc, Kelco division), and 'Gel-Gro' (ICA Biochemicals). Agar is the most commonly used gelling agent for preparation of solid and semi-solid media. It contributes to the matrix potential, the humidity and affects the availability of water and dissolved substances in the culture containers (Debergh, 1983) [5]. Various brands and grades of agar are available commercially, which differ in the amounts of impurities, and gelling capacity. Agar brands vary widely in price, performance and composition. It is the actual use and experience, which ultimately determines the choice of agar brand in a specific system and for a plant species. It is usually unnecessary to use high purity agar for large-scale micropropagation; cheaper brands of agar have been successfully used for industrial scale micropropagation (Boxus, 1978). The use of liquid media eliminates the need of agar and other options include white flour, laundry starch, semolina, potato starch, rice powder, bulla and sago. For micropropagation of ginger, pineapple, banana and turmeric, the combination of certain gelling agents gave growth as good as on agar-based media. The use of laundry starch, potato starch and semolina in a ratio of 2:1:1 reduced the cost of gelling agent by 76% (Prakash, 1993).

Source of water

Water is the main component of all plant tissue culture media. Usually in tissue culture research, distilled or doubled distilled and de-ionized water has to be used. Distilled water produced through electrical distillation is expensive. In some cases, alternative water sources can be used to lower the cost of the medium. If tap water is free from heavy metals and contaminants, it can be substituted for distilled water. Tap water has been used for *in vitro* propagation of banana (Ganapathi *et al*., 1995) [9] and ginger, *Zingiber officinale* (Sharma and Singh, 1995) [14]. Table bottled water from the supermarket can also be used a 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 changeover 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*).

Carbon sources

In tissue culture media, sucrose which is commonly used as carbon source at a concentration of 2-5% W/V, other carbohydrates are also used. Meanwhile, to reduce the tissue culture cost other sugar supplements such as sugar cane molasses, banana extract and coconut water were added to the already prepared media. The substrates in addition to sugars, they are sources of vitamins and inorganic ions required growth (Dhamankar, 1992; Zahed, 2000) [7, 17]. (Vasil & Thorpe, 1998) [16]. 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 (Demo *et al.* 2008) [7]. Sucrose has been reported as a source of both carbon and energy (Bridgen, 1994) [3]. There are reported success in reducing 90 percent cost of tissue culture banana plants by replacing sucrose. Adoption of this protocol can empower farmers to set up low cost tissue culture laboratories in their localities to increase banana plant production. Moreover, in plant propagation medium (Kaur *et al.* 2005) [10] substituted sucrose with table sugar which reduced the cost of medium considerably by 82 percent.

Acclimatization

The hardening off experiment has to be performed using different substrates such as top soil, vermin compost and sand soil in different composition of unsterilized soil media prepared at nursery. The locally soil media composition of substrates containing top soil, vermin compost, sand soil in 0:1:2 ratios gave the highest survival percentage which was 99.6% after month incubation in the primary acclimatization. The primary and secondary acclimatized seedlings have been exposed to an open field environment where they showed rapid growth after the acclimation period in greenhouse on nine months. This system is presently being introduced for the large-scale production and cost effective of this improved pineapple cv. smooth cayenne.

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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