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REGULAR ARTICLE

EFFICIENT USE OF TEMPORARY IMMERSION BIOREACTOR (TIB) ON PINEAPPLE (*Ananas comosus* L.) MULTIPLICATION AND ROOTING ABILITY

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ABSTRACT

Pineapple (Ananas comosus L.) is one of the most potential fruit crop growing in Ethiopia due to suitable agro-ecology and economic importance. However, it is difficult to meet the demand for planting materials using the conventional propagation techniques due to production inefficiency and disease transmission. The experiment is laid in Completely Randomized Design with three treatments of vessels used along its media type, Temporary Immersion Bioreactor, TIB (RITA®, Vitropic, France) , Glass jam jar and plastic jars , replicated six times on MS medium. A highly significant difference ($p<0.001$) was observed between culturing vessels with the same media supplement. From this study, it was found that pineapple explants cultured on TIB having full strength MS media supplemented with 2 mgl^{-1}BA and 30gl^{-1} sucrose was found to be better which gave an average multiplication of 13.17 shoots per explant within six weeks of culture. Similarly plantlets cultured on TIB with half strength MS media supplemented with 3mgl^{-1} IBA and 40 g l^{-1} sucrose developed on average 16.33 roots having 6.27 cm length with well developed hairy root in four weeks of culture period that performed better in acclimatization facility and open field too.

Key words: *Ananas comosus* L.; micropropagation; Temporary Immersion Bioreactor (TIB); growth regulators

INTRODUCTION

Pineapple (*Annanas comosus* L.), a member of the botanical family Bromeliaceae, is a perennial herb native to the American tropics **(Bartholomew *et al*., 2003)** which is well known for its freeness from harmful phytochemicals **(Mateljan, 2007)**. In Ethiopia, pineapple grows successfully around Gojeb, Jimma, Mizan, Bebeka, Teppi and Dilla areas. So far, yielding potential of 60 tones/ha was recorded under Gojeb condition **(Edossa, 1998)**. Except in some areas like Dilla, where the Red Spanish type is grown, the major pineapple cultivar grown in Ethiopia is Smooth Cayenne. Pineapple is largely vegetatively propagated since sexual reproduction is rare in nature due to its nature of self sterility; seeds if produced by self fertilization germinate slowly with low vigor and young seedlings are fragile due to inbreeding depression **(Bartholomew *et al*., 2003)**. So that the use of suckers, arising from buds below the ground level, slips, which are borne on the peduncle, just below or at the base of the fruit or crowns on top of the fruit are the most common ones **(Atique *et al*., 2003)**.

However, then came the issue of planting material demand, which had long been the major bottleneck towards a large-scale cultivation of the crop in Ethiopia. Different possible solutions were hence forwarded to avert the problem, including undertaking the expansion program step by step using the propagules from the existing plantations, and also to undertake *in vitro* propagation. *In vitro* propagation of pineapples for plantlet regeneration **(Kiss *et al*., 1995; Firoozabady and Gutterson, 2003)** and conservation **(Souza *et al*., 2006)** is well documented. It has comparative advantage over the traditional methods as it leads to the production of large numbers of disease-free uniform planting materials in a relatively shorter period independent of the season. The *in vitro* technique is found to be more efficient to overcome the problem of planting material shortage to attain the targeted extensive pineapple plantation. But still the efficiency of the propagation is not satisfactory using glass jars in semi solid media by restricting the level of subcultures in order to maintain the quality of the plantlets. Further increase in shoot multiplication has been achieved by the use of different type of temporary immersion system which is not automatic **(Escalona *et al*., 1999)** and bioreactors **(Firoozabady and Gutterson, 2003)**.

Bioreactors are the most promising way of scaling up micropropagation processes, since it enables a high degree of control over culture conditions (pH, aeration rate, oxygen, ethylene and carbon dioxide concentrations) and are compatible with the automation of micropropagation procedures **(Hervé Etienne *et al*., 2006)**. So far different types of bioreactors were tested on pineapple for different purposes. A few assays were carried out on

microcuttings in temporary immersion bioreactors, but the multiplication rates obtained, whilst higher than those achieved on semi-solid medium, were not sufficient to fill the gap for planting material demand that shows the need for further assessment.

Therefore the objective of this study is to assess the potential of temporary immersion bioreactor (TIB) as a vessel for pineapple in vitro shoot multiplication and rooting ability in order to increase the multiplication efficiency and rooting without compromising quality.

MATERIAL AND METHODS

Genetic Materials

The most worldwide accepted pineapple variety, Smooth Cayenne, known for its fresh consumption in addition to their canning quality was brought form horticulture department of Jimma Agricultural Research Centre, Ethiopia.

Preparation of Explants

The stock plants for the study were raised using selected healthy and uninjured pineapple plantation. The slips were thoroughly washed with local liquid soap followed by 30 minutes fungicide treatment (Mancozeb 80% W.P.). To avoid the remnants of the fungicide, the explants were thoroughly washed with sterile and distilled water five times. The inner most sheaths from the slips were used as a source of explant for *in vitro* culture.

Slip size of about 30 mm length from excised explants were thoroughly washed with sterilized distilled water and liquid soap, briefly immersed in a 70% ethanol solution according to **Abebe *et al*. (2009).** Subsequently, the explants were treated with 5% active chlorine concentration of the commonly used surface sterilization chemical, local bleach (Berekina) followed by sodium hypochlorite for 15 and 5 minutes respectively under aseptic condition. To enhance the efficacy of the sterilant chemicals, two drops of Tween-20 (as a wetting agent) was added with the addition of. The explants were thoroughly rinsed (3-4 times) with sterile distilled water to remove remnant chemicals from explant surface.

Culturing and their maintenance

Slips of 20 mm approximate length were cultured on conditioning media in a test tube after few scale sheaths were removed. MS basal medium **(Murashige and Skoog. 1962)** supplemented with 20 g l^{-1} of sucrose and 3 g l^{-1} Phytagel^{TM} (SIGMA) at 5.8 pH was used for a month as conditioning media. Then after, for multiplication stage full MS media supplemented with 2 mgl^{-1} N^6-benzyladenine (BA) and 30 gl^{-1} sucrose were tested to multiply the established cultures from conditioning media using TIB (RITA®, Vitropic, France), glass jam jar and plastic jars. After six weeks of culture, explants with proliferating shoot clumps were sub-cultured into the same fresh medium for further multiplication. After six weeks of culture period, shoots were transplanted into TIB, glass jam jar and plastic jars for rooting with half strength MS media supplemented with 3 mgl^{-1} indole-3-butyric acid (IBA) and 40 g l^{-1} sucrose. All cultures were maintained in air conditioned growth room at 60-70% relative humidity and 25 ± 2^0C with a 16 hours photoperiod from cool white 40 watt florescent bulbs under 2000-3000 lux light intensity. After *in vitro* rooting of the plantlets, they were subjected to red sheath cloth cover to acquire more radiation in the range from 610 to 720 nm for strong photosynthetic activity by chlorophyll and carotenoids absorption **(Taiz & Zeiger, 2004).**

After the plantlets developed roots and sufficiently elongated within four weeks, it were removed from the containers and planted in conical shaped seedling trays filled with a pre-sterilized potting mix of top soil, sand and well decomposed coffee husk at a 2:1:1 ratio **(Abebe et al., 2009)**. Here the seedling trays are covered with red sheath nets in order to facilitate the quality and quantity of light coming in contact with the seedlings. Subsequently the plantlets were planted in a one liter polyethylene sleeves followed by field plantation that give good result and performance at both nursery and field (Figure 3).

Measurements and data collection

Shoot multiplication data (number and length of shoot, number of leaves, fresh and dry weight of plantlets) and root data (number and length of roots) were collected after six and four weeks of culture periods respectively. Fresh weight of plantlets were determined by removal of the medium, washed in distilled water and dried with filter paper before measuring at digital sensitive balance. Dry weight was taken after drying the plantlets in dry oven at a temperature of 65–70°C for 48 hours at constant reading according to **Ayenew *et al*. (2012).**

Data analysis

The data was analyzed according to **Montgomery (2005)** using SAS, statistical software package (Version 9.1) and mean values were compared using the procedure of REGWQ (*Ryan-Einot-Gabriel-Welsch Multiple Range Test*).

RESULTS AND DISCUSSION

In vitro shoot multiplication of pineapple

The use of different types of vessels with its media to be liquid or semi solid showed highly significant difference (P<.001) for shoots number, length and the associated fresh and dry weight of the plantlets. This is inline with the finding of **Kacar et al. (2010)** that observed the type of culture vessel and lid effects on the gaseous composition inside the vessel as well as light penetration. In turn, the vessel affects growth parameters, such as shoot elongation, proliferation and fresh weight, as well as hyperhydric degradation processes.

In line with this, the use of TIB provided an average 13.17 shoots with average length of 8.67 cm followed by plastic jar derived plantlets (9.83) (Table 1). This finding is inline with **Goldfarb *et al*. (1991)** and **Madhulatha *et al*. (2004)** that observe liquid pulse treatments of growth regulators (in particular 6-benzylaminopurine) could promote regeneration from plant cell culture through organogenesis or 'somatic embryogenesis.

This result is in line with the finding of **Hervé Etienne *et al*. (2006)** for coffee propagation that explained TIB could provide a controlled culture conditions including pH, aeration rate, oxygen, ethylene and carbon dioxide concentrations. This effect has been associated with the increased accessibility of cultured cells to the growth regulators, compared to the impeded diffusion of growth regulators in solidified media **(Maene and Debergh 1985; Feito *et al*. 2001)**.

Though use of TIB gave significantly better result for both fresh (1.13 g) and dry weight (0.076 g), plastic and glass jam jar performed similar result (Figure 1 and Table 1). This could be due to similar semi solid media apart from TIB associated with nutrient translocation and air flow.

Table 1 *In vitro* shoot and root development of Pineapple by using different types of vessels

Vessels	Shoot number	Shoot length (cm)	Leaf number	Root number	Root length (cm)	Fresh weight (g)	Dry weight (g)
TIB*	13.17±1.72a	8.67±1.02a	10.17±1.83a	16.33±1.03a	6.27±0.75a	1.13±0.23a	0.076±0.015a
Plastic jar	9.83±1.94b	6.15±0.87b	10.67±1.9a	9.98±1.80b	3.22±0.33b	0.36±0.14b	0.024±0.009b
Glass jam jar	6.33±1.03c	4.33±0.66c	9.00±2.80a	9.33±2.16b	2.57±0.88b	0.16±0.09b	0.013±0.005b
P. value	<.001	<.001	0.4322	<.001	<.001	<.001	<.001
CV	16.49	13.56	22.36	14.55	17.17	29.94	27.15

Means with the same letter in a column are not significant and are separataed using the procedure of REGWQ (*Ryan-Einot-Gabriel-Welsch Multiple Range Test*).

*TIB: Temporary Immersion Bioreactor

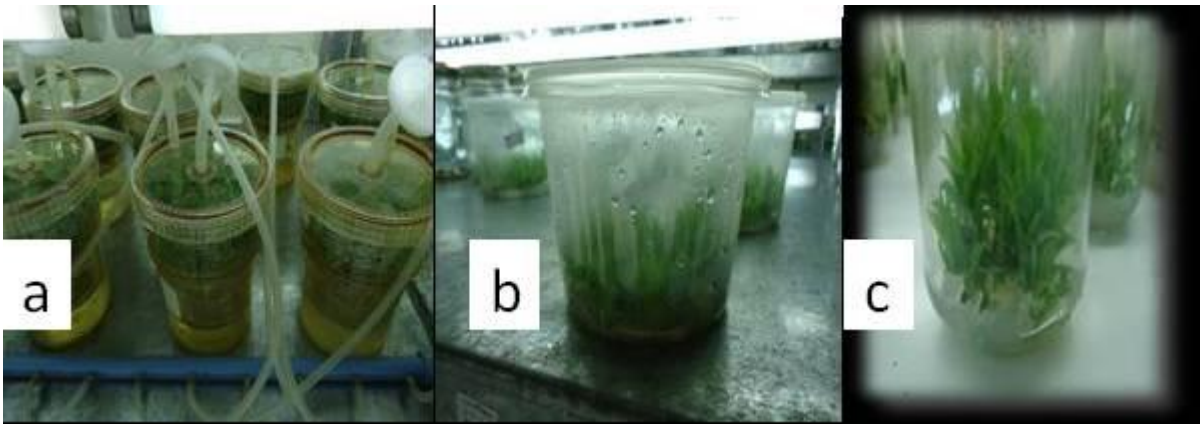

Figure 1 *In vitro* shoot multiplication of pineapple on(a)TIB (b)Plastic jar and (c)Glass jam jar

***In vitro* rooting of pineapple**

In vitro root development has an impact for the *ex vitro* acclimatization potential of the plantlets and subsequent field growth **(Danso *et al*. 2008)**. Therefore, in this study it was found that the use of different types of vessels have significant effect on rooting (P<.001). The use of TIB resulted in an average 16.33 roots with a length of 6.27 cm (Figure 2 and Table 1). Besides root number and length, plantlets derived from TIB posses numerous root hairs that could help them to absorb nutrients better which are due to contact provided between entire explants and the liquid medium. This is in line with the observation of

(Escalona *et al*. 1999) and in contrary to **Danso *et al*. (2008)** on pineapple rooting that observed no root development by using shaker based liquid media in Erlenmeyer flasks.

TIB culture combines the advantages of both solid and liquid medium. Solid cultures allow aeration but not provide full contact with nutrient media whereas Liquid medium permits an efficient nutrient uptake even though hyperhydricity is often presents **(Smith and Spoomer 1995; Aitcken-Christie et al. 1995)**. However, hyperhydricity has been never reported in pineapple liquid cultures and was not present in our automated micropropagation system due to short time exposure for the media.

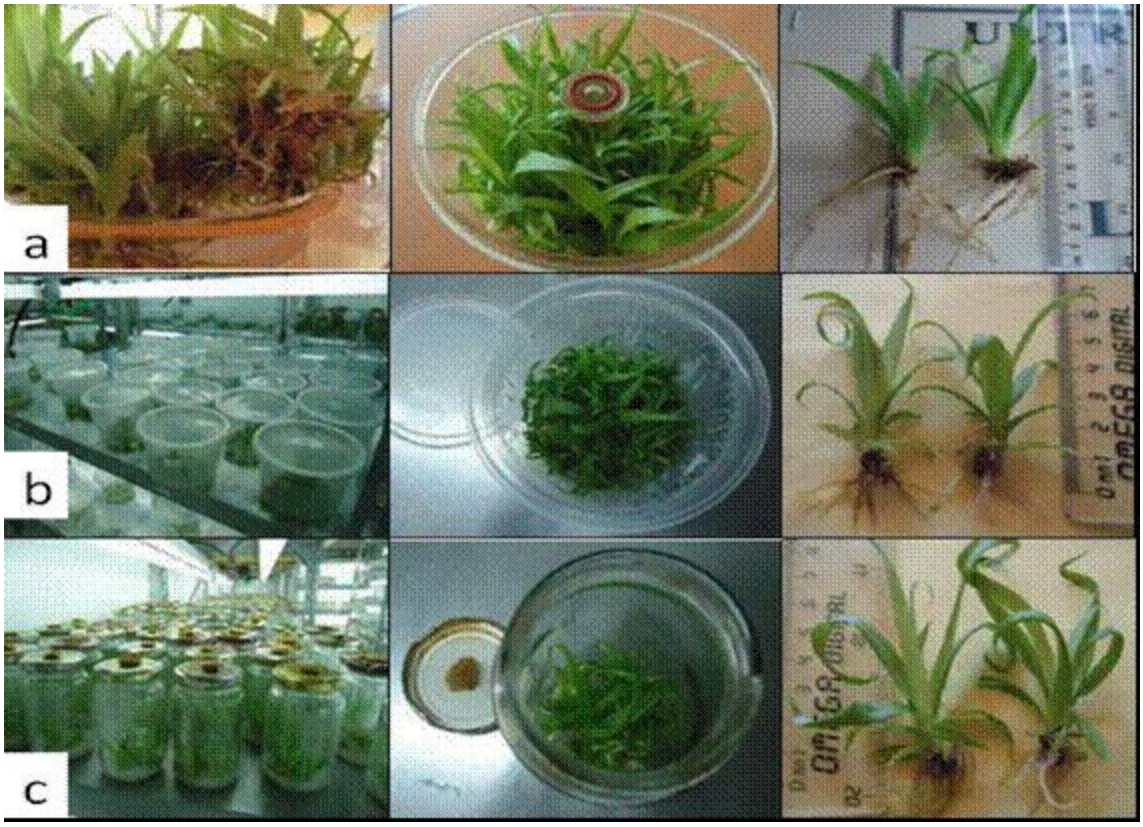

Figure 2 *In vitro* root development of pineapple on (a) TIB (b) Plastic jar and (c) Glass jam jar

***Ex vitro* acclimatization of *in vitro* raised pineapple plantlets**

Seedlings derived from TIB performed better and fast in acclimatization, this could be due to the availability of more number of root and root hairs. Since the seedlings obtained from TIB are found to be delicate than the one derived from semisolid, the first stage of acclimatization supplemented with red sheath cloth is found important and effective. Similarly, **Scaranari *et al*. (2009)** found on banana acclimatization by reducing the time of

acclimatization by the use of red cloth due to better transmittance in the Photosyntheticaly Active Radiation.

It was observed that, the seedlings showed variable performance based on the type of media mix used and the plastics containers. This indicates that further work should be done on acclimatization of pineapple seedlings on variable media and/or containers used.

Figure 3 *ex vitro* gradual performance of tissue culture grown seedlings of pineapple

CONCLUSION

The technique described here are a promising method of propagation of pineapple to a larger scale. In order to make it more effective further experiments should be done at the stage of acclimatization using different types of soil media combinations and/or acclimatizing vessels.

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