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Full Length Research Paper

Comparison of liquid culture methods and effect of temporary immersion bioreactor on growth and multiplication of banana (Musa, cv. Dwarf Cavendish)

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Four different liquids, as well as solid culture methods used in shoot propagation of banana were compared. Treatments studied were solid medium (A), liquid medium with immersion of plants (B), liquid medium with cotton culture support (C), liquid medium aerated by bubbling (D), and liquid medium with a temporary immersion bioreactor system (TIB) for 20 min every 1 h (E). After 4 weeks of culture, shoots in liquid medium with immersion and liquid medium aerated by bubbling showed none too little proliferation. Shoots in the solid medium and those cultured in liquid medium containing cotton culture supported played multiplication rates of 2.7 to 3.5 with the highest multiplication rate (> 7.00) observed in the explants that were subjected to the TIB in the medium. Three treated groups differed in the accumulation of dry matter; the lowest weight (around 0.6 g) was observed in treatments B and D, while 2 to 4 times greater accumulation was observed in the explants in the solid medium and those cultured in the liquid medium with a cotton culture support. The highest multiplication rates and weight gains were observed in the liquid medium with a TIB (E). Shoots in liquid medium continuously aerated by bubbling, displayed hyperhydricity of the outer leaf sheaths. However, this was not observed with temporary immersion of explants.

Key words: Banana, micropropagation, dwarf cavendish, temporary immersion bioreactor (TIB).

INTRODUCTION

Use of liquid medium for *in vitro* micropropagation is often described as a way of reducing both the cost of plantlet production and subculturing time of explants, in that explants do not require positioning in the medium but are simply placed in contact with it.

The advantages of liquid media in enhancing the shoot propagation (Harris and Manson, 1983; Alvard et al., 1993; Levin et al., 1999), growth (Snir and Erez, 1980) and somatic embryogenesis (Jones and Petolino, 1988; Harrel et al., 1994; Liu et al., 1998; Hosokawa et al., 1998) have been reported for several plant species. However, the use of liquid media can lead to the problem of asphyxia in explants as a result of immersion. The most commonly used preventive methods are based on the principle of partial immersion of explants to ensure aeration. Inserted absorbent substances are used to maintain contact between the medium and the lower part of the explant (filter paper, cellulose, cotton, etc.), or a depth of medium is used to enable partial emergence of the explant tissue. Direct oxygenation of the medium by bubbling could also be used in micropropagation (Preil, 1991).

The purpose of the present study was to compare the effect of different liquid culture methods on banana [cv. Dwarf cavendish (group AAA)] plants. The culture method based on temporary immersion of explants was also evaluated.

MATERIALS AND METHODS

Plant Material

Proliferation of banana cv. dwarf cavendish was achieved by splitting shoot–clusters established from a sucker shoot-tip. Shoot

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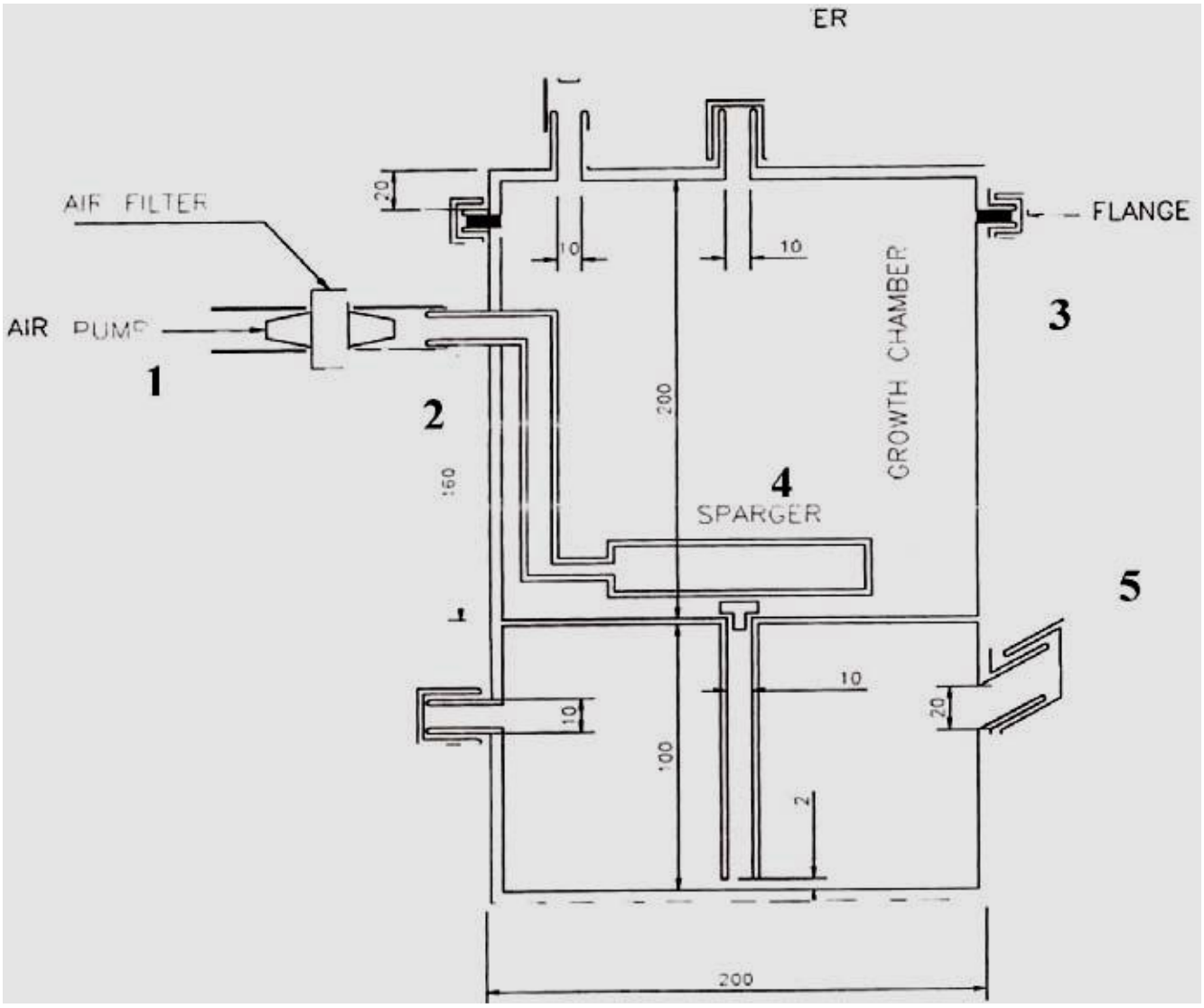

Figure 1. Vessel culture used for culturing plantlets in liquid medium aerated by bubbling 1, 22 µm filter; 2, air entrance tube; 3, upper compartment; 4, aerated heliconoid sparger; 5, extranet tube.

clusters obtained after successive proliferation subcultured on solid medium were used as initial material for the experiments. These clusters were splitted to keep 2 to 3 shoots per explant. Explants measuring approximately 1×1×1 cm with a fresh weight of 0.4 g were inoculated into the medium. Effects of treatments on the rate of proliferation and the increase in dry weight were evaluated after a 6-week subculture cycle.

Conditions of the culture

The culture medium consisted of MS medium (Murashige and Skoog, 1962) containing, 30 g/L sucrose and supplemented with 3 mg/L 6- benzyladenine and 2 mgL^{-1} indoleacetic acid (Bhagyalakshmi and Singh, 1995). Solid medium contained 6 gL^{-1} agarose (Sigma, St. Louis, USA). The pH was adjusted to 5.7 before autoclaving at 121°C and 100 kPa for 20 min. Cultures were incubated at 28°C under cool white fluorescent light (50 µmol/m^2/s^1) with a 16 h photoperiod.

Treatments

Growth of the explants during a proliferation subculture cycle on solid medium culture (treatment A) was compared with growth on various types of liquid media. The treatments used were: treatment B, immersion of explants in liquid medium; treatment C, liquid medium with cotton culture support; treatment D, liquid medium aerated by bubbling humidified air through a fritted glass filter; treatment E, liquid medium with temporary immersion bioreactor (TIB) of the explants for 20 min every 1 h. In treatment B, explants were immersed to over three-quarters of their volume as a result of surface tension, resulting in an almost total covering of the explants by the medium. In treatment C, only the base of explants was in contact with the medium. In treatment D, the aeration system involving bubbling of the medium is shown in Figure 1. In treatment D, air was continuously circulated by an air pump and passed through a sterile filter, liquid medium was aerated by the air-pump, bubbles were initiated in vessels and explants were in contact with liquid medium aerated by bubbling. In treatment E (TIB), the two

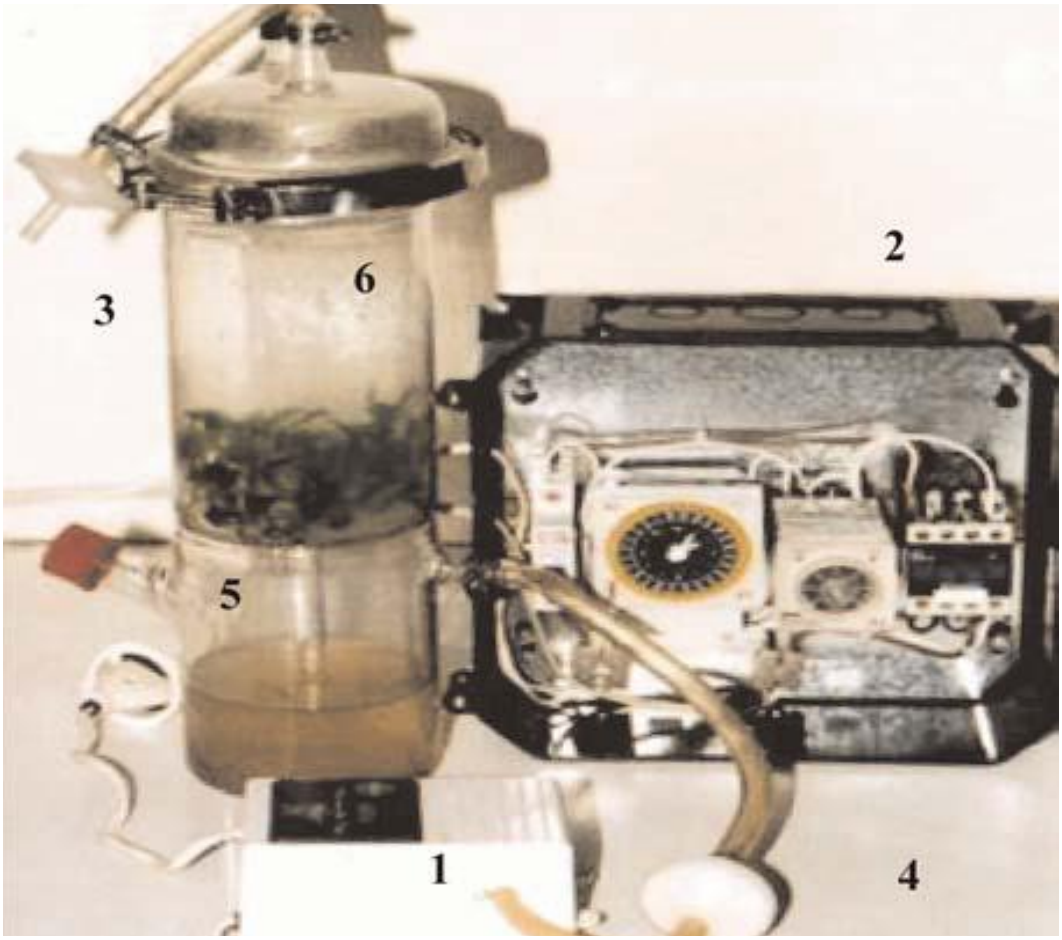

Figure 2. Vessel used for liquid culture with temporary immersion of the plantlets. 1, air pump; 2, timer set; 3, vessel culture; 4, 22 µm filter; 5, tube connecting the upper and lower compartments of the culture vessel; 6, upper compartment with plants.

compartments were connected by a tube and each was fitted with a 0.2 µm filter vent. The explants were placed in the upper compartment, and the lower compartment contained the nutrient solution (Figure 2). Putting the lower compartment under pressure by means of an air pump caused the culture medium to rise into the upper compartment and the immersed liquid was released through the filter vent in the upper compartment. The medium flowed back by gravity when the pump was switched off. The medium return flow to the lower compartment was accelerated by fitting a solenoid valve in the air circuit to enable a return to atmospheric pressure when the pump was stopped. The quantity of medium used in treatments A, B, C was 25 ml and in treatments D and E, was 100 ml and 250 ml, respectively.

Measurements and analysis of the results

The explant multiplication rate was calculated by the ratio of the "number of shoots at the end of the subculture cycle''/ "initial number of shoots''. The dry weight of explants at the end of subculture was determined. Three culture vessels each containing 5 proliferation clumps were used in each treatment. The averages of the 3 replications are presented. The differences between treatments were evaluated using the ANOVA test ($P < 0.05$), and curves were drawn with the SPPS soft ware version 10.

RESULTS AND DISCUSSION

Multiplication rate

After 6 weeks of culturing, three treated groups were found to be different in their multiplication rates (Figure 3). The banana shoots clusters in the liquid medium with immersion or aerated by bubbling showed very little proliferation. Those grown on solid medium and subcultured in liquid medium with cotton substrate had multiplication rates of 2.7 to 3.5 and explants growing in the TIB had the highest rate of 7 (Figure 4).

Dry weight gain

Measurement of dry weight separated the treatments into three groups (Figure 5). The lowest dry weights (0.4 to 0.8 g) were recorded in treatments B and D; moderate dry weights were in treatments A and C and this was increased by 2 to 4 times in the B and D treatments. The highest dry weight was observed in treatment E (TIB)

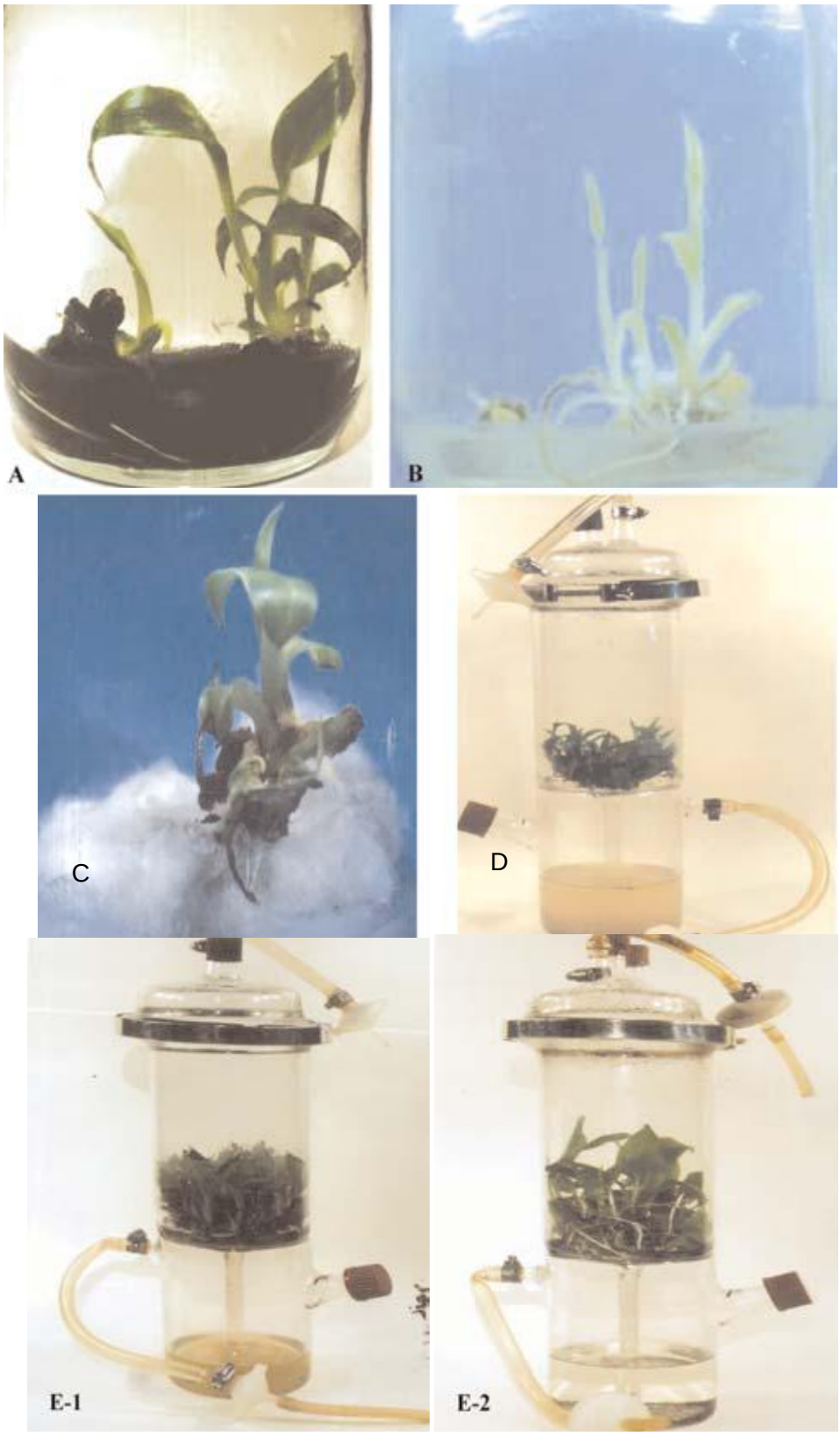

Figure 3. Comparison of solidified and liquid media culture methods with respect to banana multiplication rate. A, solid medium; B, simple liquid medium and total immersion of plants; C, liquid medium and cotton support; D, liquid medium and aeration by bubbling; E, liquid medium and temporary immersion bioreactor (TIB) of plant (1) Micropropagation (2,) growth.

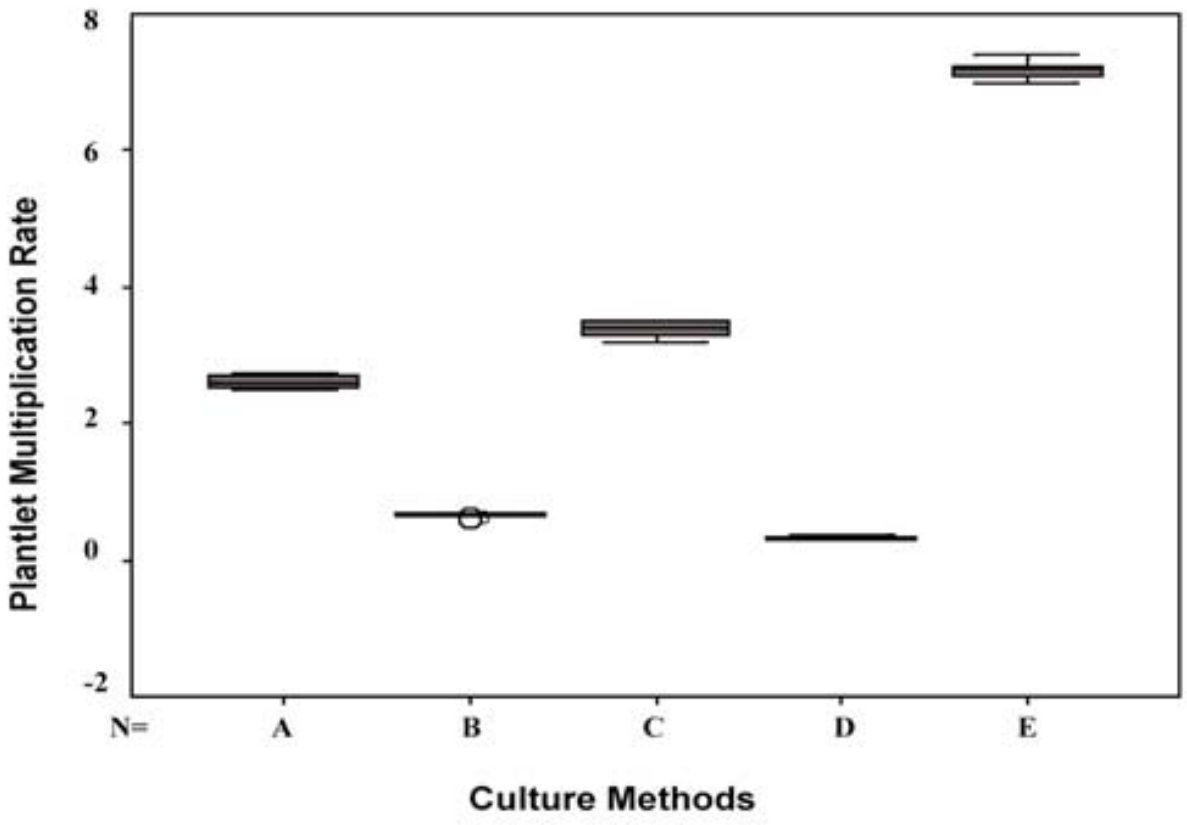

Figure 4. Plantlet multiplication rates in different culture methods A, solid medium; B, simple liquid medium and total immersion of plants; C, liquid Medium and cotton support; D, liquid medium and aeration by bubbling; E, liquid medium and temporary immersion bioreactor (TIB) of plants.

(3.2 to 3.4 g).

Appearance of *in vitro* shoots

Explants in liquid medium with simple immersion and liquid medium aerated by bubbling (treatments B and D) displayed numerous necrotic zones and lacked leaves. Very small leaves appeared in shoots in liquid medium with cotton support (treatment C). Several external leaf sheaths in treatment D showed hyperhydricity and these leaves were sometimes fragile. Leaf development was considerable in the temporary immersion bioreactor. By comparing liquid and aerated liquid medium, roots grown in liquid medium are shown to be under hypoxic stress. Roots grown in a bubbling aerated system, were not hypoxic, but produced low biomass.

Plantlets propagated in TIB showed better performance than those propagated by conventional methods such as micropropagation (Gonzales-Olmedo et al., 2005; Perez et al., 2004; Ilezuk et al., 2005).

In the method of increasing the biomass level in the culture (Scragge, 1995) plantlets seem to use more of the nutrients of the medium than their photoassimilates (Escalona et al., 2003). These results showed that the response of tissues to the gas phase composition are complex and requires further study (Weathers et al., 1999). These experiments showed that the type of liquid medium application greatly influences the development of banana explants in micropropagation. Up to a four-fold difference in proliferation and accumulation of dry matter was observed with the same medium composition. The photosynthetic capacity and the main enzymatic systems related to carbon metabolism, changed during the *in vitro* culture of plantain shoots (Musa AAB cv. CEMSA ¾), in temporary immersion bioreactor and their subsequent acclimatization (Aragon et al., 2005). The relationships between morphological parameters, photosynthetic capacity of the plantlets and enzymes of carbon metabolism during both phases of the culture have been shown previously (Escalona et al., 2003). Differences in growth between the explants in simple liquid medium without aeration (immersion medium, treatment B), liquid medium aerated by bubbling (treatment D) and with TIB (treatment E) suggested that the lack of oxygen in the liquid media containing small explants and asphyxia of explants as a results of immersion, are the major limiting factors to growth. Banana explants in a simple liquid medium, are immersed to over three-quarters of their volume. Surface tension caused most of the explants to

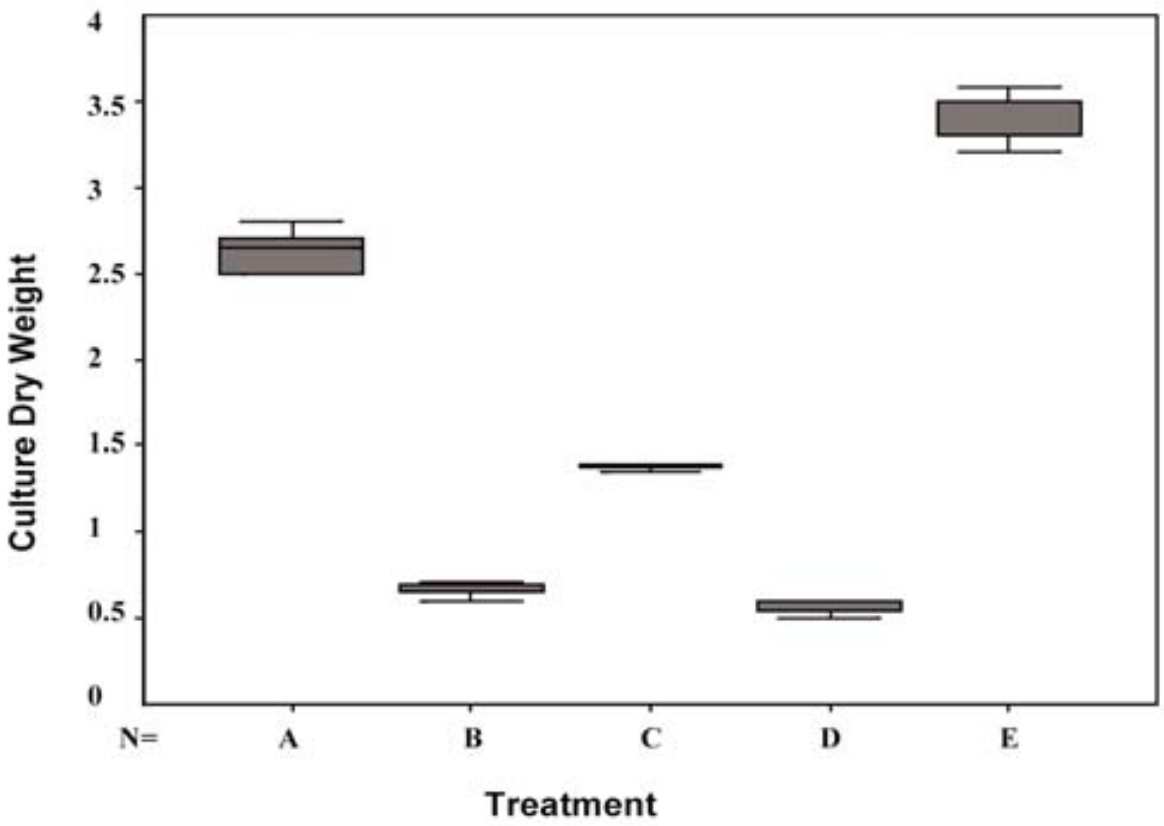

Figure 5. Comparison of the effect of different forms of liquid medium application on the increase in dry matter in banana micropropagation. A, solid medium; B, simple liquid medium and total immersion of plants; C, liquid medium and cotton support; D, liquid medium and aeration by bubbling; E, liquid medium and temporary immersion bioreactor (TIB) of plants.

be covered by the medium. Lack of culture agitation apparently led to asphyxiation of the explants. The low proliferation rate observed in banana, cultured on cotton supports (treatment C) cannot be ascribed to the problem of oxygenation since small parts of each explants were immersed. However, the small surface area of explant in contact with the medium-soaked support may have limited water supply, a carbohydrate and mineral nutrition which may be the reason for the weak development and continuous contact of explants with the medium in treatment D may be the cause of the hyperhydricity observed. Vuylsteke (1989), reported the highest rates of proliferation in several cultivars of bananas and also the type of temporary immersion culture used for the propagation of *Pinus* (Aitken-Christie and Jones, 1987), *Echinacea angustifolia* (Lata et al., 2004), strawberry, pear, apple (Damiano et al., 2003), *Eucalyptus, Aspen* and *Psidium* (Ruffoni and Savona, 2005). Alvard et al. (1993) used a type of temporary immersion culture system for the propagation of banana cv. Grand Naine. Our system has also been successful in potato micropropagation, by singlenodes and microtubers production, by a considerable reduction of the immersion time (Zarghami and Ebadi, 2001). Akita and Takayama (1994) also succeeded in the stimulation of potato tuberization. The duration of explant immersion is probably the fact that deserves the most attention in design of culture systems in liquid medium with temporary immersion for other species to be propagated *in vitro*. The temporary immersion culture system (TIB) described combines the ability to aerate plant tissue and provide contact of programmable duration, between the whole explant and the medium. In addition, the system used in this study had the decisive advantage of being easy to set up and use. Storage of the culture solution and explant immersion were performed in the same vessel with no external mechanical transfer system. This simplicity of use should make it easier to test the temporary immersion method for improvement of the *in vitro* development of other species or for developing automated culture systems. Methods of automation can reduce the cost, scaling-up in micropropagation can increase the number of explants handled, thereby decreasing the labour costs (Takayama and Akita, 1994). This can be achieved in several different ways: (1) Homogenisation of plant tissue in blenders rather than

manual cutting, (2) Automation through use of liquid cultures and bioreactors, and (3) Robotics (Eide et al., 2003).

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REFERENCES

Aitken-Christie J, Jones C (1987). Toward automation: radiate pine shoot hedges *in vitro*. Plant Cell Tissue Org. Cult. 8: 185-196.

Akita M, Takayama S (1994). Stimulation of potato (*Solanum tuberosum* L.) tuberization by semicontinuous liquid medium surface level control. Plant Cell Rep. 13: 184-187.

Alvard D, Cote F, Teisson C (1993). Comparison of method of liquid medium culture for banana micropropagation, effects of temporary immersion of explants. Plant Cell Tissue Org. Cult. 32: 55-60.

Aragon EC, Escalona M, Capote I, Pina P, Cejas I, Rodriguez R, Canal MJ, Sandoval J, Roels S, Debergh P, Gonzalez- Olmedo J (2005). Photosynthesis and carbon metabolism in plantain (Musa AAB) plantlets growing in *ex vitro* acclimatization. *In Vitro* Cell Dev. Biol. Plant. 41: 550-554.

Bhagyalakshmi N, Singh S (1995). Role of liquid versus agargelled media in mass propagation and *ex vitro* survival in bananas. Plant Cell Tissue Org. Cult. 41: 71-73.

Damiano C, Monticelli S, La Starza SR, Gentile A, Frattarelli A (2003). Temperate fruit plant propagation through temporary immersion. XXVI International Horticultural Congress: Biotechnology in Horticultural Crop Improvement: Achievements, Opportunities and Limitations 1(56), ISBN 9066052589, ISSN 0567-7572.

Eide AKH, Munster C, Heyerdahl PH, Lyngved R, Olsen S (2003). Liquid culture systems for plant propagation. 1(56), ISBN 9066052589, ISSN 0567-7572.

Escalona M, Samson G, Borroto C (2003). Physiology of effects of temporary immersion bioreactors on micropropagated pineapple plantlets. *In vitro* Cell Dev. Biol. Plant. 39: 651-656.

Gonzales-Olmedo JL, Fundora Z, Molina LA, Abdulnour J, Desjardins Y, Escalona M (2005). New contributions to propagation of pineapple (Ananas comosus L. Merr) in temporary immersion bioreactors. *In Vitro* Cell. Dev. Biol. Plant. 41: 87-90.

Harris RE, Mason EBB (1983). Two machines for *in vitro* propagation of plants in liquid media. Can. J. Plant Sci. 63: 311-316.

Harrel RC, Bieniek M, Hood CF, Munilla R, Cantliffe DJ (1994). Automated, *in vitro* harvest of somatic embryos. Plant Cell Tissue Org Cult. 39: 171-183.

Hosokawa K, Oikawa Y, Yamamura S (1998). Mass propagation of ornamental gentian in liquid medium. Plant Cell Tissue Org. Cult. 17: 747-751.

Ilczuk A, Winkelmann T, Richartz S, Witomska M, Serek M (2005). *In vitro* propagation of Hippeastrum × chmielii Chm.- influence of flurprimidol and the culture in solid or liquid medium and in temporary immersion systems. Plant Cell Tissue Org. Cult. 83: 330-346. 316.

Jones AM, Petolino JF (1988). Effects of support medium on embryo and plant production from cultured anthers of soft-red winter wheat. Plant Cell Tissue Org. Cult. 12: 253-261.

Lata H, Bedir E, Moraes RM, Andrade Z (2004). Mass propagation of Echinacea angustifolia: a protocol refinement using shoot encapsulation and temporary immersion liquid system. XXVI International Horticultural Congress: The Future for Medicinal and Aromatic Plants 1(68), ISBN 9066055073, ISSN 0567-7572.

Levin R, Alper Y, Stav R, Abed A, Watad A (1999). Methods and apparatus for liquid media and semi-automated micropropagation. Hort. Biotech. *In Vitro* Cult. Breed. pp. 659-663.

Liu CZ, Wang YC, Guo C, Ouyang F, Ye HC (1998). Production of Artemisia by shoot cultures of *Artemisia annua* L. in a modified inner-loop mist bioreactor. Plant Sci. 135: 211-217.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-497.

Perez A, Napoles L, Carvajal C, Hernandez M, Lorenzo C (2004). Effect of sucrose, inorganic salts, inositol, and thiamine on protease excretion during pineapple culture in temporary immersion bioreactors. *In Vitro* Cell Dev. Biol. plant, 40: 311-315.

Preil A (1991). Application of bioreactors in plant propagation In: Debergh PC 7 Zimmerman RH (Eds) Micropropagation: Technology and Application. Kluwer Academic Publisher, Dordrecht. pp. 426-445.

Ruffoni B, Savona M (2005). The temporary immersion system (T.I.S.) for the improvement of micropropagation of ornamental plants 1(59), ISBN 9066056584, ISSN.0567-7572.

Scragge AH (1995). The problem associated with high biomass levels in plant cell suspension. Plant Cell Tissue Org. Cult. 43: 163-170.

Snir I, Erez A(1980). *In vitro* propagation of Malling Merton apple rootstocks. Hort. Sci., 15: 597-598.

Takayama S, Akita M (1994). The types of bioreactors used for shoots and embryos. Plant Cell Tissue Org. Cult. 39:147-156.

Vuylsteke DR (1989). Shoot-tip culture for the propagation. Conservation and exchange of Musa germplasm. International Board for Plant Genetic Resources, Rome, pp. 1-59. ISBN 92-043-40-7.

Weathers PJ, Wyslouzil BE, Wobbe KK, Kim YJ, Yigit E (1999). Of hairy roots to O2 levels in bioreactor. *In Vitro* Cell Dev. Biol. Plant. 35: 286-289.

Zarghami R, Ebadi M (2001). Stimulation of potato (*Solanum tuberosum* L.) tuberization by semicontinuous liquid medium. Horticultural Congress: Iranian society for Horticultural Sciences Karaj -Iran 1(361).