Chapter 8

Cost-effective mass cloning of plants in liquid media using a novel growtek bioreactor

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Abstract: A low-cost Growtek bioreactor has been designed, patented and commercialised. It has unique features such as a floating and rotating explant-holder with perforated explant support and a side tube for medium changing, culture feeding and for content monitoring. The bioreactor can be operated both in static and agitated modes. Extensive performance studies have been conducted using representatives of trees (*Santalum album*), commercial ornamentals (*Dendranthema grandiflora*), monocotyledonous horticultural species (*Ananas comosus*), tuber crops (*Solanum tuberosum*) and a medicinal plant (*Catharanthus roseus*). In comparison to propagation in agar-gelled media as well as in liquid media using other culture vessels, this bioreactor exhibited 1.2 – 23.3 times shoot production, minimised root injuries by 32 – 48 %, reduced contamination by 12 – 18 % and reduced incubation time by 16- 42 %. Thousands of *Ananas comosus* plantlets raised in this bioreactor have been field tested. Additionally, it was found to be effective for hairy root culture of *C. roseus*.

Key words: Chrysanthemum, Catharanthus, cost-effectiveness, Growtek bioreactor, liquid medium, mass cloning, pineapple, potato, Santalum

Abbreviations: BAP- 6-benzylaminopurine; GA- gibberellic acid; GI- growth rate; IAA- indole-3-acetic acid; IBA- indole-3-butyric acid; MS- Murashige and Skoog’s (1962) medium; NAA- naphthalene acetic acid

1. Introduction

The industrial production of tissue cultured plants has largely been dominated by herbaceous ornamental species and a few vegetable, fruit or plantation crops (banana, oil palm etc.). The success with woody and semi-woody plants has been rare (Smith, 1997; Gupta et al., 1993). The high cost
of production (~ US$ 0.10 – 0.15 per unit) owing to the labour-intensive nature (labour cost may be 50-85 % of production cost), prejudicing economic viability, was the single most important reason that discouraged in vitro industrial propagation of many species (Vasil, 1994; Goldstein, 1999). Plant tissue culture was practised initially with agar-gelled media. It was soon realized that agar was one of the costliest ingredients in the medium, though not a nutrient. Many gelling and non-gelling matrices were tested in order to achieve cost-effectiveness, by substituting agar (Sorvari, 1986; Henderson and Kinnersley, 1988; Bhattacharya et al., 1994). Subsequently, the use of liquid media, scale-up in bioreactors (Preil, 1991; Takayama, 1991; Das et al., 1999) and induction of automated production were some of the alternatives explored for the minimization of cost of production through improvement in propagation efficiencies (Tisserat, 1991; Smith and Spomer, 1995; Hvoslef-Eide and Melby, 2000; Dey, 2001). The prospects for temporary immersion have also been discussed (Etienne et al., 1997; Jimenez et al., 1999). The other aspects of cost minimisation are the use of low-cost culture vessels, prevention of contamination, improved quality of plantlets and their enhanced field survival. The successful adaptations of these alternatives may also enhance the scope for commercial exploitation of somatic embryogenesis, plant secondary metabolite production (Curtis and Emery, 1993; Hunter and Kilby, 1999) and heterologously-expressed healthcare products of human origin (Doran, 2000; Meyer et al., 2002). The recent attempts at the production of such new generation products as plantibodies (Peeters et al., 2001; Stoger et al., 2002) are indicators of further need for developing the most cost-effective bio-processes based on plant cell and tissue culture in liquid media. The use of liquid media in these cases will offer benefits of increased nutrient uptake, greater availability of dissolved oxygen, easier dispensing, automated scale up and process control, periodic sampling and more productivity.

Our laboratory has been working for more than a decade on cost-minimisation aspects through novel bio-process (Indian patent application No. 197/Cal/2001), product (Bhattacharya et al., 1994) and equipment development. This article describes the performance of the novel Growtek bioreactor for mass cloning of several commercially-important plants.