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# *Aloe barbadensis* Mill. Ex vitro Autotrophic Culture

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## ABSTRACT

*Aloe plants can be grown autotrophically in a maintenance-free, sterile, ex vitro environment. Excel, 15-5-15 plus minors fertilizer formulation and Plant Preservative Medium (PPM) biocide was used as a very economical basal medium. In vitro micropropagated plantlets were regenerated in Excel media with 2 g /L sucrose and 2.2 µM 6-Benzylamino purine (BA) and used to initiate ex vitro cultures in open vessels. Ex vitro media loss due to evaporation was reduced by sealing the culture vessel around the plantlets with either parafilm or aluminum foil and by adjusting gellan gum levels. Parafilm and higher levels of gellan gum significantly reduced ex vitro media loss but higher gellan gum levels (6 g /L or 8 g /L) also reduced plant weight gains compared to plants grown in 4 g /L gellan gum for 25 weeks after transplanting.*

KEYWORDS: *Aloe barbadensis*, in vitro propagation, micropropagation, plant tissue culture

## INTRODUCTION

Aloe vera (barbadensis Miller)has been used for centuries and is currently being actively studied for medicinal purposes (Grindlay and Reynolds, 1986; Davis *et al*., 1994; Heggers *et al*., 1993; Shelton 1991; Visuthikosol *et al*., 1995). Common names include "Medicine Plant," "Burn Plant," "First Aid Plant," and "Miracle Plant." Egyptian Manuscripts describe the many medicinal values of aloe including applications for treating kidney ailments, blistering, hair loss, sunburn, constipation, stomach disorders, skin care, itching, headaches and hemorroids (Skousen, 1979). Today, it is also believed to possess anti-cancer agents. The aloe vera gel is comprised of a mixture of antibiotics, astringent and coagulating agents. Other compounds inhibit pain and scar development while stimulating growth of new tissue. These healing properties have made this plant an effective optional treatment for human ulcers, including peptic, dendrite karatitis and cutaneous leishmaniasias.

The bioactivity of the medicinal compounds in the gel becomes questionable when the gel is processed. Unfortunately, it is impractical for hospitals and care units to have available fresh, unprocessed material because of cultivational requirements.

The major objective of this study was to create an autotrophic (culture medium without a carbon and energy source such as sucrose) culture growth system for growth of aloe plants in a clean, economical and maintenance-free medium (Kozai, 1989, Kozai *et al*., 1987, Fujiwara *et al*., 1987). The plants would be grown under artificial lighting and ex vitro conditions (not in completely enclosed culture vessel).

## MATERIALS AND METHODS

### Culture of in vitro plants

Field plants were transferred into black cinder : peat moss mixtures (3:1) and grown in the greenhouse. Shoot tips of new growth were used as initial explants. Outer leaves from the shoot tips were

removed until only the youngest pair of leaves remained. Shoot tips were soaked in Exspor disinfectant (4 parts water : 1 part base : 1 part activator) for 30 minutes (Tanabe *et al.*, 2001). Explants were further reduced in size, soaked in sterile water for 1-2 mins and transferred into Excel culture medium (15-5-15 plus minors) with 2 ml/L Plant Preservative Medium (PPM). Media treatments included 2.50 g/L Excel plus the following combinations: 20 g/L sucrose + 0 $\mu$M BA, 20 g/L sucrose + 2.2 $\mu$M BA, 0 g/L sucrose + 0 $\mu$M BA, 0 g/L sucrose + 2.2 $\mu$M BA. Cultures were maintained at 22° C under a 16 hr photoperiod provided by Philips cool-white fluorescent tubes, at a light intensity of 50 $\mu$mol $m^{-2}s.^{-1}$

**Culture of ex vitro plants**

Media combinations with different levels of gellan gum were tested with either aluminum foil or parafilm as vessel sealing materials. The following media combinations were placed into 3" x 2.5" plastic tubs: 2.5 g/L Excel + 4 g/L gellan gum + 2 ml/L PPM; 2.5 g/L Excel + 6 g/L gellan gum + 2 ml/L PPM; 2.5 g/L Excel + 8 g/L gellan gum + 2 ml/L PPM. Heavy duty aluminum foil or parafilm were used to seal the vessels. A slit was made through the sealing material and the base of micropropagated plants passed through the slit and pushed into the media. Cultures were maintained under similar light and temperature conditions as the in vitro cultures.

Weekly media weight loss was determined as a per cent of weight loss by comparing original weight to weight at the end of each week. Weekly plant weight gain or loss was determined as a per cent of original plant weight.

## RESULTS AND DISCUSSION

Sucrose in the culture medium influenced the rate of contamination. Treatments without sucrose showed no contamination 1 week after explanting, whereas, treatments with 20 g/L sucrose showed 42.9% and 75% contamination during this same period. Treatments without sucrose continued to show less contamination than sucrose containing media until the 4$^{th}$ week. Since most contaminants rely on sucrose for carbon and energy, growth of these contaminants will be limited when sucrose is absent. Latent contamination development may be a result of the release of carbon containing compounds from the explants.

Media containing BA and sucrose promoted shoot growth. A medium combination with 2 g/L sucrose + 2.2 $\mu$M BA showed regeneration of a new shoot in 80% of its cultures in 6 weeks. Exclusion of BA or sucrose resulted in regeneration in 40% or less of the cultures. Although BA plays an important role in shoot initiation, it seems that sucrose is also involved in shoot initiation and/or shoot development.

Culture medium loss was a concern because it could influence the chemical integrity of the medium. A faster rate of medium loss would require earlier and more frequent subcultures.

Parafilm was more effective in reducing culture medium weight loss (Fig. 1). An average of 1.5% weight loss occurred with parafilm after 14 weeks as compared to 4.0% weight loss with aluminum foil. Both materials are water impervious, but parafilm provides a tighter seal.

Gellan gum influenced culture medium loss (Fig. 2). Media with 4 g/L gellan gum had an average weekly media loss of 9.5%. Whereas, media with 6 g/L and 8 g/L, showed losses of 5.5% and 6.0% respectively. Total media losses after 25 weeks for media with 4 g/L, 6 g/L and 8 g/L gellan gum were 53%, 33% and 34% respectively.

Gellan gum is used as a solidifying agent, therefore, treatments with less gellan gum tended to be more liquefied and were more prone to evaporation and weight loss.

Gellan gum levels influenced ex vitro plantlet weight gains. Plantlets grown in media with 4 g/L gellan gum had an average weight gain of 74%. Cultures with 6 g/L and 8 g/L gellan gum showed 13% weight gain and 5% weight loss respectively (Fig. 3). Media with more gellan gum probably influenced the plantlets ability to absorb water and other compounds due to the highly solidified nature of the media.

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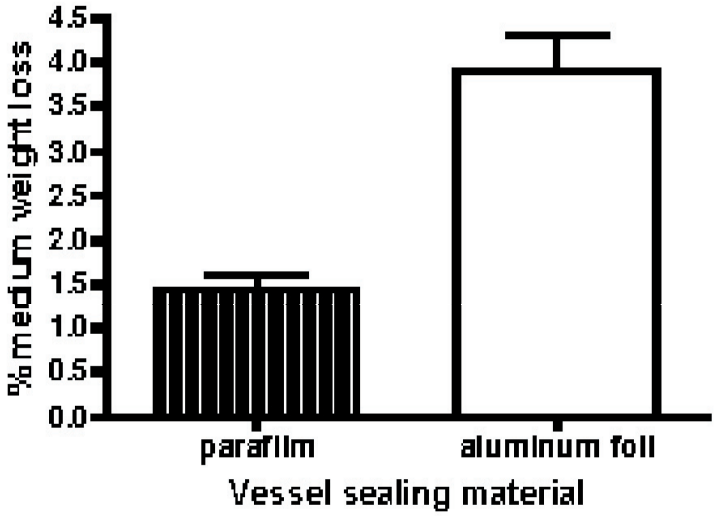


**Fig. 1. Media weight loss of ex vitro cultures sealed with parafilm and aluminum foil after 14 weeks. Data are mean ± SE.**

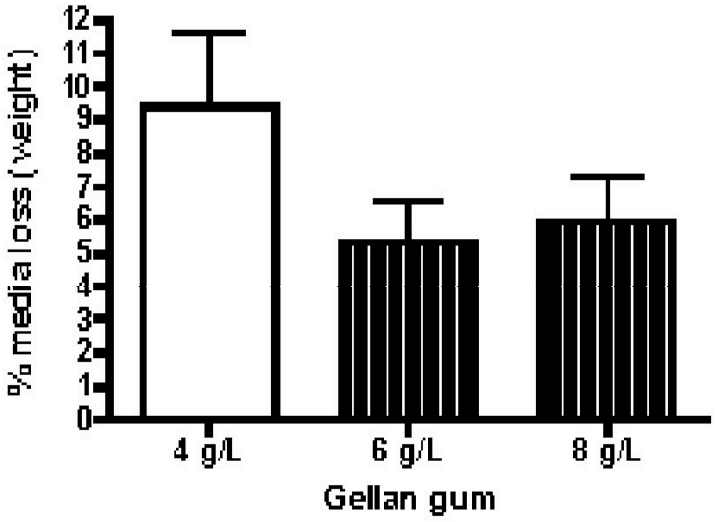


**Fig.2. Effect of gellan gum on the average weekly ex vitro media weight loss. Data are mean ± SE.**

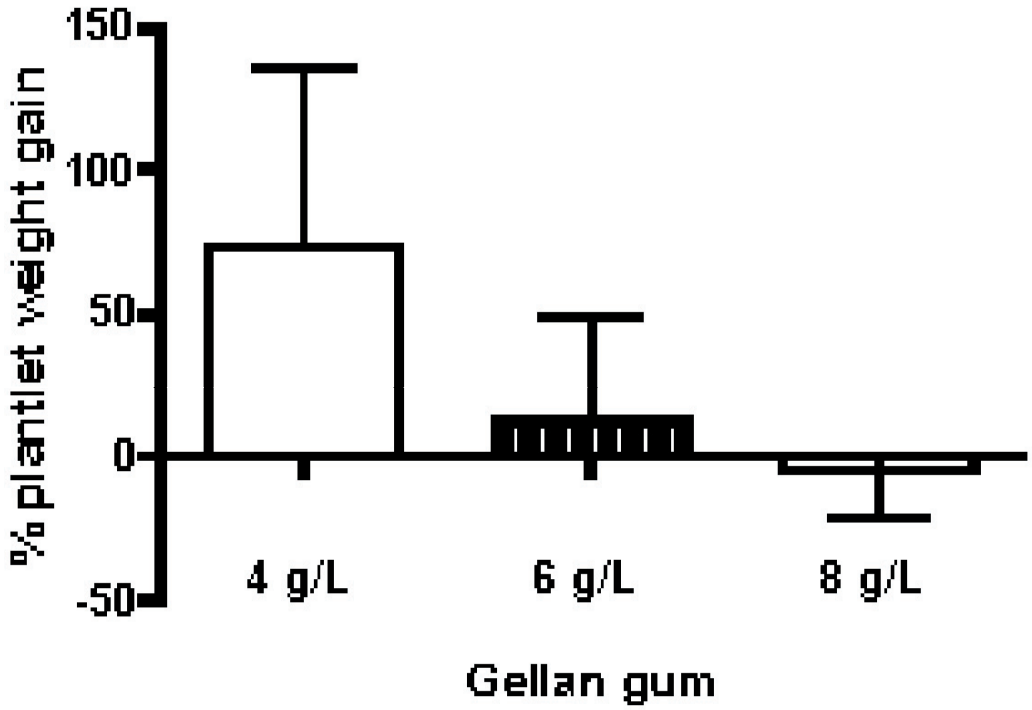


**Fig. 3. Effect of incorporation of gellan gum into the medium on ex vitro plantlet weight gain at 25 weeks. Data are mean ± SE.**