(restricted), thus avoiding potential landscape problems. For those still thinking that a taproot is necessary, consider which is stronger, a single steel rod or a multistrand cable of the same diameter. Remember, it is not how much real estate is moved as the rootball, but rather what is IN the real estate.

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Micropropagation of Sweet Viburnum (*Viburnum odoratissimum*)©

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INTRODUCTION

Sweet viburnum (*Viburnum odoratissimum*, Ker-Gawl.), an evergreen shrub native from Japan and the Himalayas, is widely used in Florida landscapes as a foundation plant for large buildings, borders and hedges (Dehgan, 1998). Because of its fast growth and prompt response to nitrogen fertilization, sweet viburnum is being used as a model plant to study root and shoot growth cycles and nitrogen nutrition (Martins et al., unpublished data). However, the use of non-clonal material leads to asynchronous flushes among plants. This problem has been reported not only on sweet viburnum but also on cacao (Greathouse et al. 1971), lychee (Marler and Willis, 1996), and oak (Borchert, 1975). Borchert (1973) suggested the use of clonal material to overcome this problem.

Sweet viburnum can be vegetatively propagated by cuttings (Dehgan, 1998), however, in vitro propagation (micropropagation) techniques could be applied to produce physiological uniform clonal plants in a relatively short time period. Nobre et al. (2000) developed a successful protocol for in vitro propagation of *V. tinus*, a Mediterranean viburnum species, however, there is no report of in vitro propagation of *V. odoratissimum*.

Another advantage of using in vitro plants in growth flush studies is that shoot or root flushes can be easily controlled using appropriate growth regulators, therefore, facilitating studies of the effects of growth flushes on nutrient uptake. The objective of this study was to determine the feasibility of developing a protocol for the in vitro propagation of *V. odoratissimum* not only to be used as an aid in studying growth cycles and nutrient uptake, but also to be used in commercial propagation.

MATERIALS AND METHODS

Softwood shoot-tip explants about 3 cm long were first collected from liners growing in a 30% shade house at the Department of Environmental Horticulture, University of Florida, Gainesville, Florida in Oct. 2000. Liners were induced to flush by applying nitrogen fertilizer and explants were collect for a second establishment in Dec. 2000. Before sterilization, leaves were removed and stems were placed under flowing tap water for 30 min. Surface sterilization was accomplished by immersion in a 50% (v/v) ethanol solution for 30 s followed by 15 min immersion in a gently agitated solution containing 1.05% sodium hypochlorite (v/v) plus 5 drops Tween-20 per 100 ml. Stems were rinsed three times in sterile deionized water and apical single node explants about 5 mm long were transferred to 150 × 25 mm glass culture tubes containing 12 ml establishment medium. The establishment medium consisted of Murashige and Skoog [MS] (Murashige and Skoog, 1962) inorganic salts and vitamins, supplemented with 2.2 μM of benzyladenine [BA], 3% sucrose and 7 $g \cdot liter^{-1}$ of TC agar (Phytotechnology Labs, Mission, KS). The medium was adjusted to pH 5.7 with 0.1 N KOH before autoclaving. Explants were subcultured monthly onto the same medium.

On 10 Jan. 2001, explants, initially established in Oct. 2000, were transferred to a medium consisting of Woody Plant Medium [WPM] (Lloyd and McCown, 1980) inorganic salts and vitamins, 3% sucrose supplemented with 4.4 μM BA and solidified with 7 $g \cdot liter^{-1}$ TC agar (Nobre et al. 2000). Shoots were subcultured monthly to this medium. Axillary shoot multiplication was successfully obtained using this medium. Because the shoots produced were short and clustered, an experiment was designed to determine the optimum growth regulator combination and concentration for both optimum shoot production and elongation. In June 2001, uniform 4-shoot-cluster explants about 4 mm long were transferred to 150 × 25-mm culture tubes containing 12 ml WPM supplemented with BA at 5 levels (0, 0.5, 1.1, 2.2, or 4.4 μM) and GA_3 at 4 levels (0, 2.8, 14, or 28 μM). A culture tube inoculated with a shoot cluster explant served as the experimental unit. Each treatment was replicated 11 times using a completely randomized design. Treatment effects on shoot production, number of shoots longer than 12 mm and length of the 3 longest shoots per culture were evaluated. The experiment was repeated once. Data were statistically analyzed using the GLM procedure (Statistical Analysis System, 1990). Statements of significant refer to $p \leq 0.05$.

RESULTS AND DISCUSSION

Stage I contamination and survival rates were influenced by developmental stage of the explants. Explants established in October consisted of floral buds while those established in December consisted of vegetative buds from flushed plants. Contamination of floral bud explants was higher (50%) than that of vegetative buds (5%), however, vegetative buds formed callus and became oxidized, leading to the death of most explants. It is conceivable that flushed plants exhibit differences in endogenous growth regulator balances leading to callus formation and phenolic exudation. Despite a high contamination rate, floral buds showed no oxidation and developed greater number of shoots than vegetative buds (Fig. 1). Similar results were found by Brand (1993), who found optimum explant collection time for deciduous woody plants to be prior to and after the period of rapid shoot elongation.

Modgil et al. (1999) reported that apple explants established during the summer

displayed the lowest contamination and higher survival rates compared to explants collected at other times of the year.

In our research, shoots produced on medium supplemented with BA alone resulted in production of short rosette shoot clusters (see Fig. 2). Often shoot microcuttings that are too small exhibit difficulties in rooting (Banko and Stefani, 1989; Polanco et al., 1988). Thus, an experiment was completed to determine the optimum

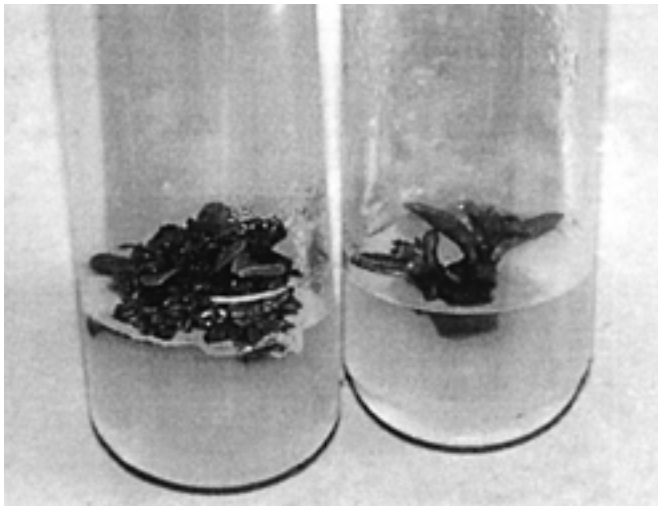

Figure 1. Differences in establishment of sweet viburnum from floral (left) and vegetative bud explants (right) in culture.

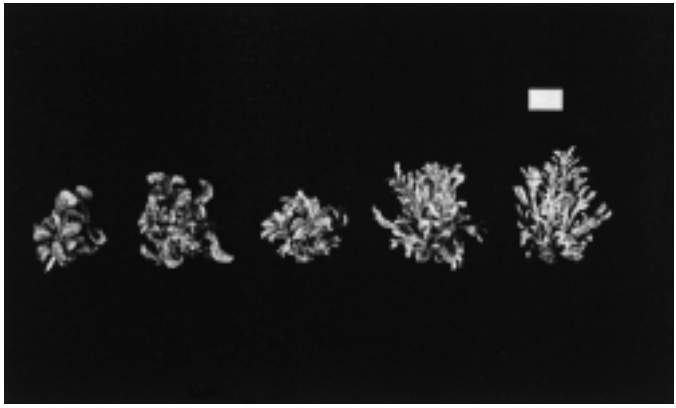

Figure 2. Effects of GA_3 concentration on production and elongation of sweet viburnum shoots in the presence and absence of 0.55 (M BA after 4 weeks culture. Treatments from left to right: 0 µM BA + 0 µM GA3; 0.55 µM BA + 0 µM GA_3; 0.55 µM BA + 14 µM GA_3; 0.55 µM BA + 28 µM GA_3. Note presence of roots on shoot cluster cultured on medium without growth regulators (left). Scale bar = 1 cm.

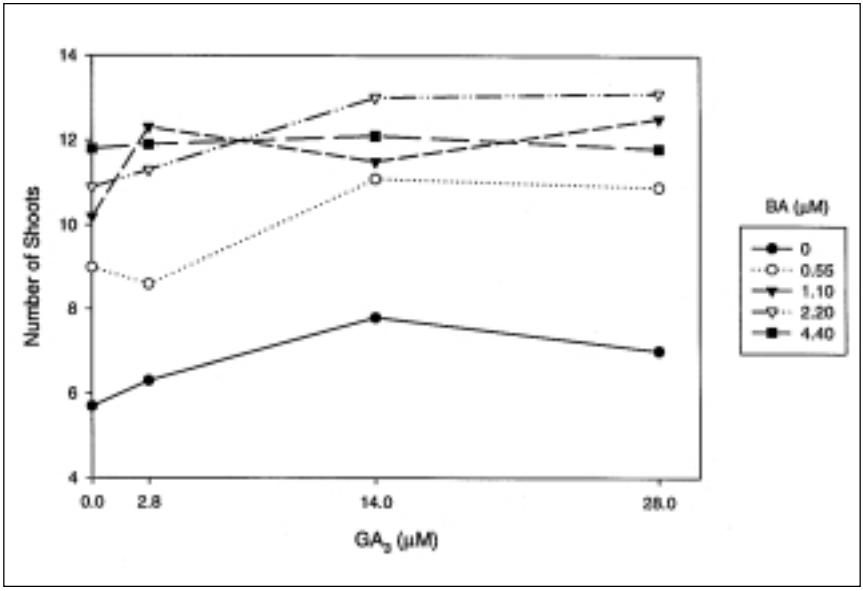

Figure 3. Effects of BA and GA3 concentration and combination on total shoot production of sweet viburnum after 4 weeks culture.

combination of BA and GA_3 to promote both shoot multiplication and elongation.

Maximum total shoot production per explant occurred in the presence of 2.2 µM BA with either 14 or 28 µM GA_3 (Fig. 3). Shoot elongation, suppressed in the presence of BA alone, was significantly enhanced by addition of GA_3 particularly at lower BA (0.55 µM) levels (Fig. 4). However, optimal production of shoots longer than 12 mm (a minimal microcutting length considered as rootable) was observed in the presence of 0.55 µM BA and 28 µM GA_3 (Fig. 5). Sahoo and Chand (1998) also reported that a combination of BA and GA_3 enhanced frequency of shoot development and promoted internode elongation in *Vitex negundo*, a woody medicinal shrub.

Explants cultured in medium containing no BA or GA_3 developed roots (see Fig. 2). Even at lower rates, BA inhibited root formation (Fig. 2). This concurs with George (1993), who suggested cytokinins inhibit or delay root formation. We have observed that microcuttings maintained in WPM without growth regulators (0 BA and 0 GA_3) root within 4 weeks. An ex vitro 60 day survival rate of about 50% was observed when these rooted microcuttings were transplanted to a peat-based medium under a 5 s/10 min intermittent mist interval (Fig.6). Further experiments are needed to determine the optimum concentration of growth regulators to induce root formation to increase plant survival ex vitro.

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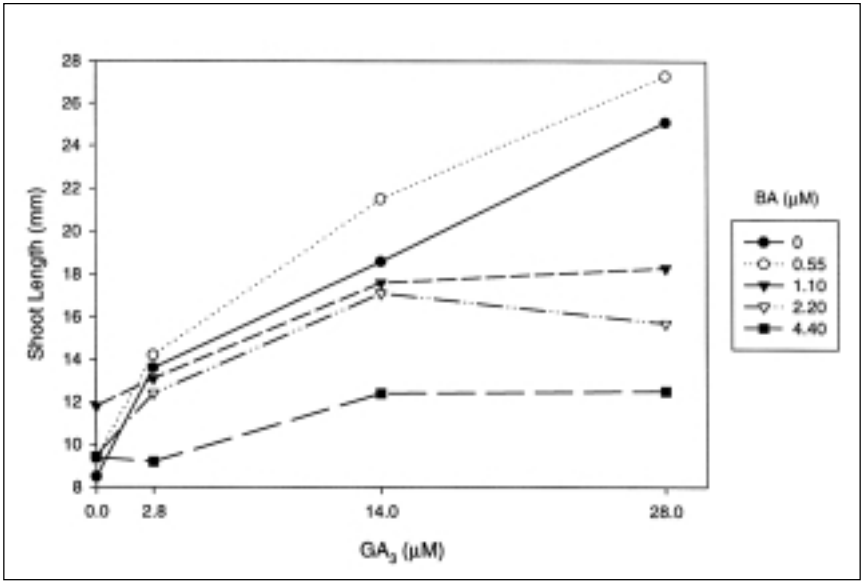

Figure 4. Effects of BA and GA3 concentration and combination on mean shoot length of sweet viburnum after 4 weeks culture.

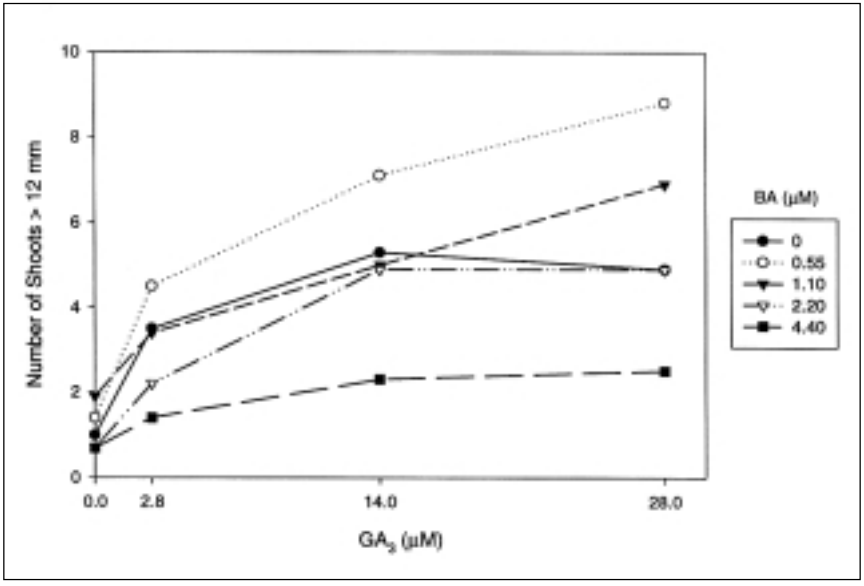

Figure 5. Effect of BA and GA3 concentrations on production of shoots > 12 mm long after 4 weeks culture.

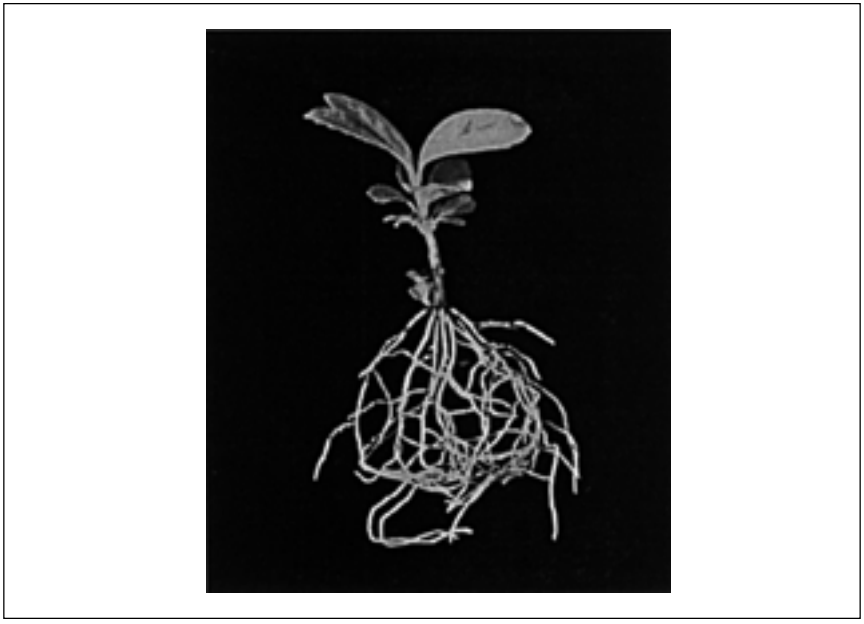

Figure 6. Acclimatized rooted sweet viburnum plantlet 60 days post-transplanting.

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