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Full Length Research Paper

Evaluation of different ratios of auxin and cytokinin for the i*n vitro* propagation of *Streptocarpus rexii* Lindl.

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A successful means of *in vitro* propagation of *Streptocarpus rexii* Lindl. using leaf tissue as the explants is described. Secondary explants were placed on several regeneration media supplemented with various concentrations of indole-acetic acid (IAA) and benzyladenine (BA). Optimal shoot proliferation was obtained on Murashige and Skoog basal media containing 0.1 mg/L IAA in combination with 0.5 mg/L BA. The growth of explants, as quantified by their fresh and dry weights, was significantly higher in the 1 mg/L IAA with 1 mg/L BA treatment as compared with the 0.1 mg/L IAA plus 1.0 mg/L BA. The increasing concentrations of BA reduced the percentage of explants forming roots. From regeneration media, shoots were transferred to Murashige and Skoog medium supplemented with 1 mg/L IAA for root induction. After six weeks, the rooted plantlets were removed from culture and successfully hardened off in the shade house. For the multiplication of *S. rexii* Lindl., the best combination was the one involving 1 mg/L IAA and BA. This treatment obtained a highest shoot count and had the highest growth rate. This could play a key role in the large scale mass propagation of this plant.

Key words: *Streptocarpus rexii* Lindl., initiation, rooting.

INTRODUCTION

The genus *Streptocarpus* belongs to the family Gesneriaceae. The genus is comprised of 132 species (Burtt and Hilliard, 1971), of which 87 species originate from South Africa, 41 species from Madagascar and the Comoro Islands and 4 species are found in Asia, which are not closely related to the African and Madagascar species (Afkhami-Sarvestani et al., 2006). *Streptocarpus* species are divided into two subgenera according to their morphological characteristics. The subgenus *Streptocarpella* (44 species) (Afkhami-Sarvestani et al., 2006) have well-developed stems, numerous small leaves and auxiliary inflorescences usually marked by having 30 chromosomes (Burtt and Hilliard, 1971). The subgenus *Streptocarpus* (88 species) show long peduncles, have 32 chromosomes and a rosette growth habit (Burtt and Hilliard, 1971; Afkhami-Sarvestani et al., 2006).

Streptocarpus rexii Lindl., occurring eastward in South Africa from George to Kwazulu-Natal (Goldblatt and Manning, 2000), was the first species of the genus to be discovered, as it occurs further to the south west than Cape Town, where botanical exploration began (Burtt and Hilliard, 1971). Introduced into cultivation from the first discovered plants, it has become by far the best known species of *Streptocarpus* and the basis of the garden strains developed by its hybridization with various other species (Burtt and Hilliard, 1971). This plant is a rosulate perennial, up to 20 cm in height, and flowers in October to April. Flowers are funnel-shaped, tube flaring, with streaks in the throat (Goldblatt and Manning, 2000). *Streptocarpus* spp. Are propagated conventionally either sexually by seeds or vegetatively by divisions and leaf

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Abbreviations: ANOVA, Analysis of variance; **BA,** benzyladenine; **cm,** centimetres; **g/L,** grams per litre; **HCl,** hydrochloric acid; **IAA,** indole-acetic acid; **MS,** Murashige and Skoog; **Min,** minutes; **mg/L,** milligrams per litre; **ml,** millilitres; **mm,** millimetres.

cuttings (Burtt and Hilliard, 1971). The *in vitro* approach can allow for more efficient and productive propagation of selected individuals than conventional propagation procedures, particularly when parent plant material is limited, since very little explants material is required for plant regeneration. The correct auxin: cytokinin ratio is important as their combination may interact and promote or inhibit the development of shoots and roots (Böttger, 1974; Kamat and Rao, 1978; Goodwin and Morris, 1979; Hinchee and Rost, 1986; Kataeva et al., 1991; Alexandrova et al., 1996; Echeverrigaray et al., 2000; Casanova et al., 2004; Bohidar et al., 2008). The objective of this study was to develop a protocol for the rapid and mass propagation of *S. rexii* Lindl.

MATERIALS AND METHODS

Initiation

Young healthy leaf material from adult specimens of *S. rexii* Lindl. were used as explants. Leaves ranging from 4 to 15 cm in length, taken from two plants were rinsed in running tap water for 15 min, surface-sterilized in a solution of 1.5% sodium hypochlorite and 0.1% Tween 20 for 10 min, and then finally rinsed three times in sterilized distilled water for 20 min each time. The leaves were then cut into 1 × 1 cm explants containing the leaf vein (Kyte and Kleyn, 1996). The leaf explants were placed into prepared glass tubes (100 × 23 mm) containing 10 ml of MS (Murashige and Skoog, 1962) medium supplemented with 20 mg/L myo-inositol, 0.2 mg/L thiamine-HCI, 1 mg/L indole-acetic acid (IAA), 0.1 mg/L benzyladenine (BA), 30 g/L sucrose and solidified with 7 g/L agar. The pH was adjusted to 5.8 prior to autoclaving (Afkhami-Sarvestani et al., 2006) for 20 min at 121°C. The culture tubes were sealed with parafilm around the edges of the caps. The cultures were maintained in a growth room at 25 ± 2°C with a 16 h light and 8 h dark cycle (Kyte and Kleyn, 1996).

Rooting

For rooting, *in vitro* shoots were excised from shoot clusters and individual leaf shoots approximately 10 × 15 mm in size were cultured individually on MS medium supplemented with 100 mg/L myo-inositol, 0.4 mg/L thiamine-HCI, 1 mg/L IAA, 30 g/L sucrose and were solidified with 7 g/L agar. The pH was adjusted to 5.8 prior to autoclaving.

Hardening off

After 6 weeks, rooted plantlets of *S. rexii* Lindl. were removed from tissue culture for acclimatization in the greenhouse. The agar medium was washed from the roots of the plantlets, before being planted in punnet trays in a moist medium of sifted bark and vermiculite (1:1). Trays were watered thoroughly and were then covered with plastic domes in the greenhouse. The domes were propped up by 7 cm on the sides after 1 week and completely removed after 2 weeks. The trays were then placed under domes of 50% shade cloth for a further 2 weeks in the greenhouse before they were transferred to an external shade house.

Multiplication

Seven weeks after initiating the cultures, the secondary explants which were leaves of approximately 10 × 5 mm in size were transferred to several regeneration media. The basic culture medium consisted of MS (Murashige and Skoog, 1962) medium supplemented with 20 mg/L myo-inositol, 0.2 mg/L thiamine-HCI, 30 g/L sucrose and were solidified with 7 g/L agar. Various concentrations of IAA and BA at the ratios of 0.1:0.1; 0.5:0.1; 1:0.1; 0.1:0.5; 0.1:1 and 1:1, respectively were added to the medium to investigate the influence of different concentrations of these growth regulators on shoot initiation and growth of *Streptocarpus* cultures. The pH was adjusted to 5.8 prior to autoclaving (Afkhami-Sarvestani et al., 2006). Twenty-five aseptic replicates were used for each treatment. The treatments were evaluated by recording the fresh mass (g), percentage explants rooted, the number of shoots initiated per explants and the dry mass (g) after drying at 60°C for 65 h. Data collected were analyzed for statistical significance using one-way analysis of variance (ANOVA). Data were presented as mean values with predicted standard errors (SE). These computations were done with the STATISTICA software program version 2010 (StatSoft Inc., Tulsa, OK, USA). The Fisher least significance difference was used to compare treatment means at P = 0.05 level of significance (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The general growth responses of leaf explants cultured on the different IAA and BA supplemented media were recorded after 12 weeks (Figure 1A to E). The analysis indicated that there were significant ($P \leq 0.05$) differences in the number of shoots formed on media with varying concentrations of auxins and cytokinins (Figure 2). The optimal concentration of obtaining the highest number of shoots was the combination of 0.1 mg/L IAA and 0.5 mg/L BA. This shoot-multiplication treatment produced 41 ± 8.42 shoots per explants. Also effective, but to a lesser degree was the 1 mg/L IAA with 1 mg/L BA treatment, producing 36 ± 6.82 shoots per explants. In other related studies, proper combination of cytokinin and auxin formulations have shown to be critical for shoot elongation in many other plant species for their *in vitro* propagation (Rout et al., 2000; Karim et al., 2003; Skala and Wysokińska, 2004; Nair and Reghunath, 2009). The correct ratio of cytokinin and auxin is important as their interaction can promote the development of shoots (Kamat and Rao, 1978). Similar to our study, a low auxin concentration in combination with cytokinin has been shown to promote root proliferation (Bohidar et al., 2008). The explants regenerated through organogenesis were removed from the test tubes and weighed. The fresh weight at harvest and the dry weight after drying at 60°C for 65 h was measured (Figures 3 and 4). The weights of explants were used to indicate the relative growth and size of explants. In all treatments, the fresh and dry weights were significantly affected by the various concentrations of IAA and BA. The concentration yielding the largest fresh and dry weights was the 1 mg/L IAA with 1 mg/L BA treatment. Thus, indicating that the growth of

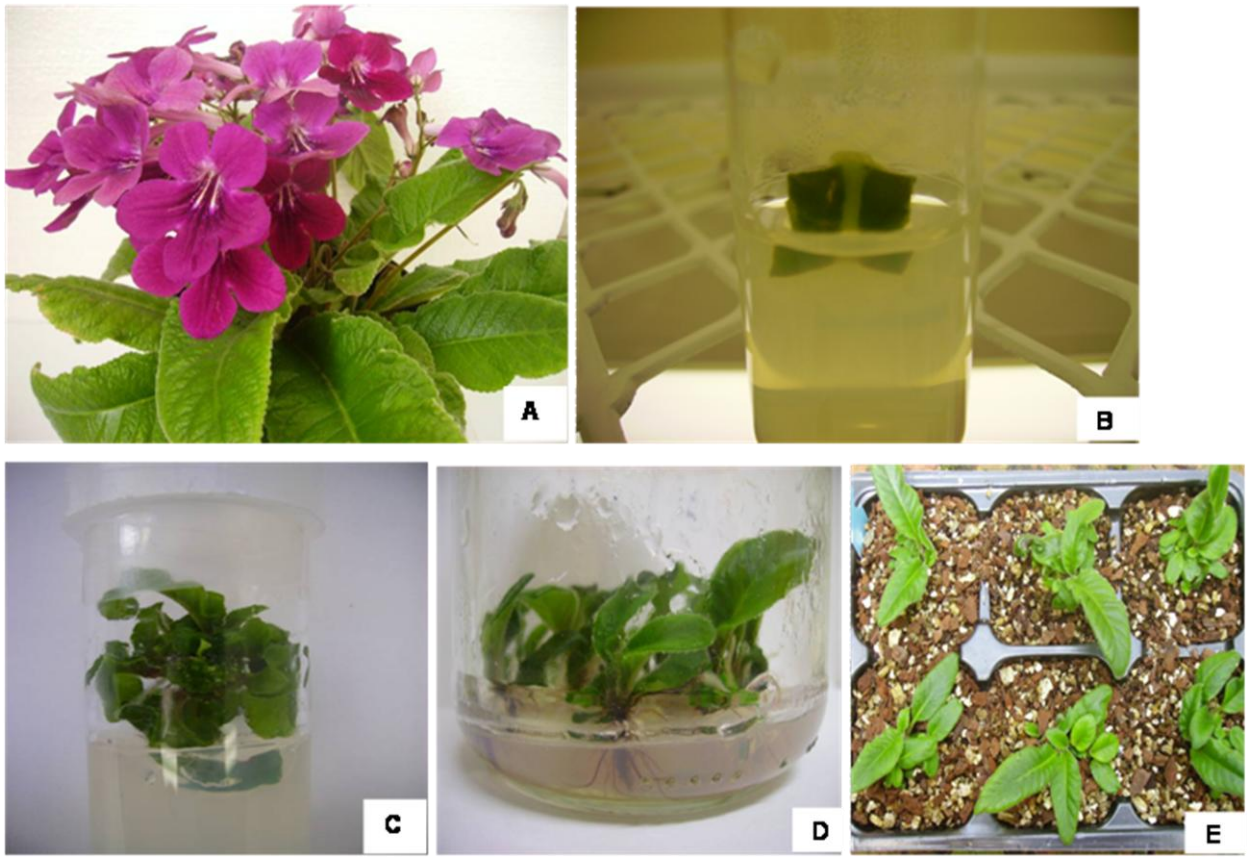

Figure 1. Stages in the micro propagation of *S. rexii* Lindl. (A) Adult plant of *S. rexii,* a source of leaf explants; (B) Excised leaf; (C) Shoot production of MS medium supplemented with 1 mg/L IAA and BA; (D) Root initiation of MS medium containing 1 mg/L IAA; (E) *Ex vitro* plants after 2 weeks in a potting medium, sifted bark:vermiculite (1:1).

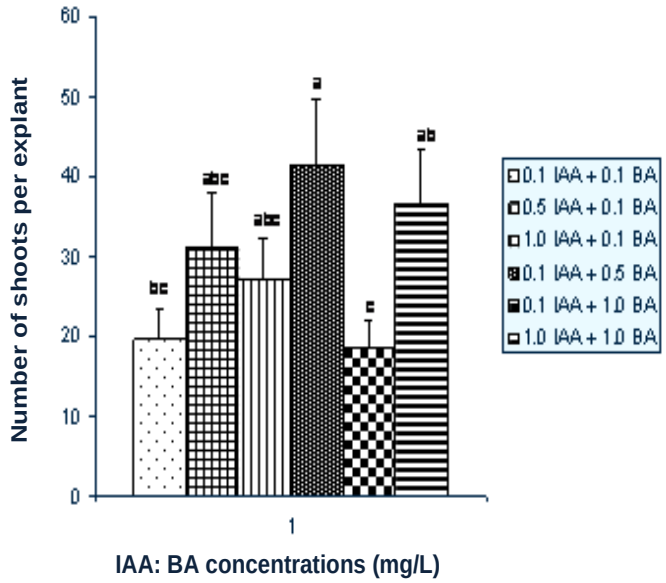

Figure 2. Effects of various concentrations of IAA and BA on the number of shoots formed per explants of *S. rexii* Lindl. after a growth period of 12 weeks. Values followed by the same letter(s) do not differ significantly p≤0.05.

explants were significantly ($P \leq 0.05$) better in this treatment. These results are in support of the hypothesis that excess of cytokinins and auxins induced rapid divisions of cells in meristimatic tissues and hence resulting into the observed better growth (Kataeva et al., 1991; Echeverrigaray et al., 2000; Casanova et al., 2004). The ratio between the number of shoots and the weight, suggests the size of shoots to be larger in this treatment. The treatment yielding the lowest weight was the 0.1 mg/L IAA plus 1.0 mg/L BA. The explants in this treatment only weighed 32% of the fresh weight and 44% of the dry weight of the highest yielding treatment.

In the multiplication stage of culture growth, the absence of roots appears to help promote shoot growth and multiplication. If roots do appear, the culture generally stops multiplying and matures (Kyte and Kleyn, 1996). Thus, the formation of roots is undesirable during the multiplication stages of culture growth. In these investigations, the treatments of higher cytokinin concentrations significantly decreased rooting percentage (Figure 5) (Böttger, 1974; Goodwin and Morris, 1979; Hinchee and Rost, 1986). In an experiment involving a treatment containing 1 mg/L BA, no root growth was

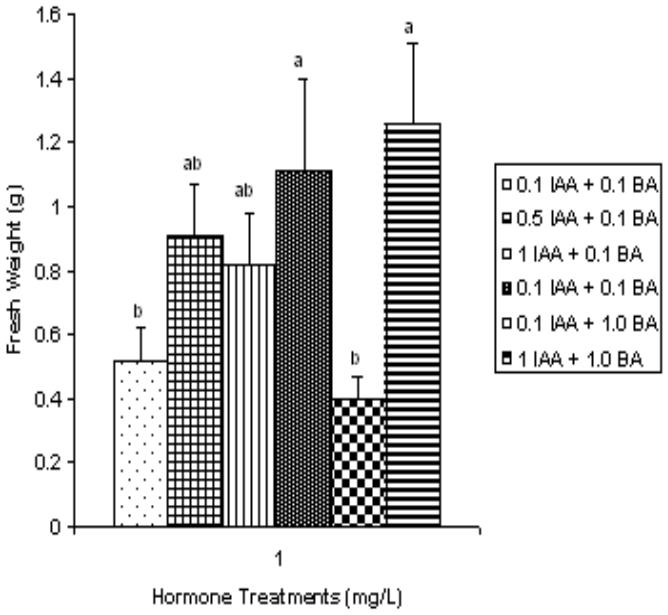

Figure 3. The effect of various concentrations of IAA and BA on the fresh weight of explants after growth period of 12 weeks. Values followed by the same letter do not differ significantly (P = 0.05).

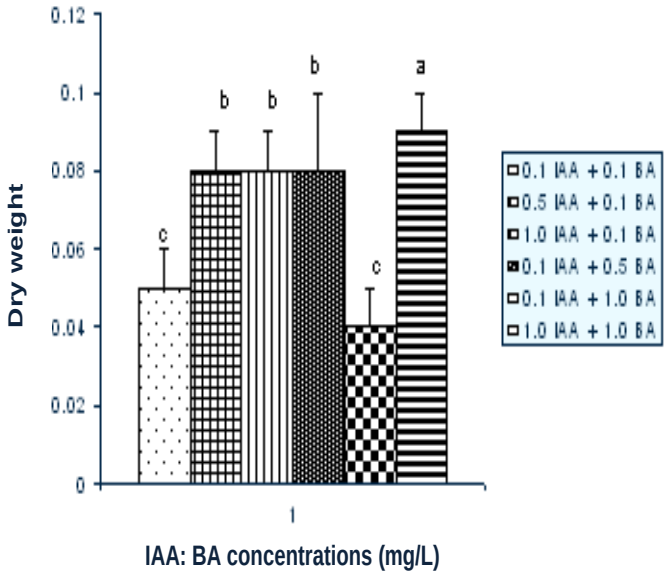

Figure 4. The effect of the various concentrations of IAA and BA on the dry weight of explants sfter a growth period of 12 weeks. Values followed by the same letter do not differ significantly (P = 0.05).

observed. Alexandrova et al. (1996) also found root formation to be suppressed on cytokinin containing media.

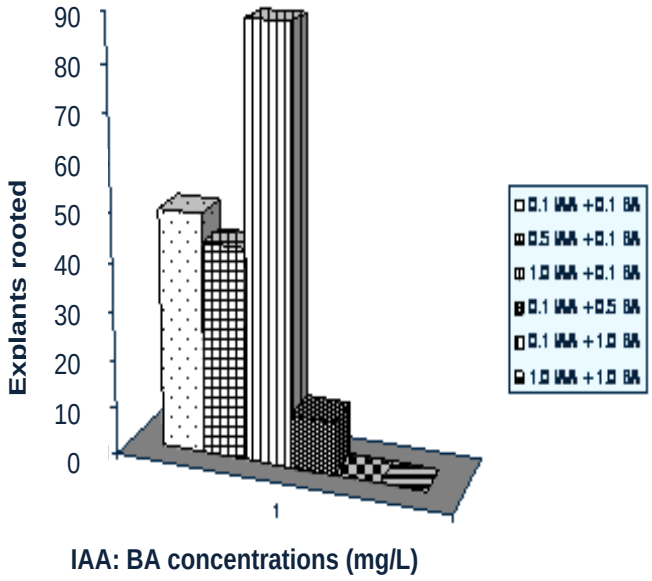

Figure 5. The effects of various concentrations of IAA and BA on the % of explants forming roots.

Conclusion

The combination of 1 mg/L IAA and BA appears to be the optimal concentration for the multiplication of *S. rexii* Lindl. This treatment obtained a high shoot count and had the highest growth rate based on the weights of the explants. Furthermore, this was the recommended auxin:cytokinin ratio as there was no root growth in this treatment, thus promoting increased multiplication. This package could play a key role in the large scale mass propagation of *S. rexii* Lindl.

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