

Effect of coconut (*Cocos nucifera* L.) water extracts on adventitious root development in vegetative propagation of *Dracaena purplecompacta* L.

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Abstract Propagation by softwood canes and cuttings is preferred as a practical system for vegetative reproduction of many ornamental plant species, despite the advances in tissue culture techniques. *Dracaena purplecompacta* L. is a species that has a high demand for exports. Conversely, coconut water (CW) is a rich supplement that naturally contains plant growth regulators such as indole acetic acid (IAA). The objective of this work was to evaluate the potential of CW extracts containing natural IAA, on adventitious root development in vegetative propagation of ornamental plant canes of *D. purplecompacta* L. Five different concentrations (28, 57, 143, 286, 571 μM of natural IAA) of CW extracts were tested. Another set of treatment was carried out with the same concentrations of authentic IAA hormone for comparison purpose. The 143- μM IAA CW extract recorded the best root induction and development. It was found that the root expression was faster (5 weeks) with the use of the novel method. In the conventional method, the canes are propagated by quick dip application of commercial product containing artificial hormone IAA and placing them on coir fiber dust beds. It takes up to 6 weeks for the canes to develop adventitious roots to the desired level. Steeping canes in 143- μM IAA CW extract improved rooting in *D. purplecompacta* L., and it was comparable to the application of 143- μM authentic IAA. The study indicates that adventitious root development, shoot development, and

leaf emergence of *D. purplecompacta* L. is promoted by IAA CW extracts.

Keywords Coconut water · Indole acetic acid · *Dracaena purplecompacta* L. · Adventitious roots

Abbreviations

| | |
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| CW | Coconut water |
| IAA | Indole acetic acid |
| IBA | Indole butyric acid |
| NAA | Naphthelene acetic acid |
| TLC | Thin layer chromatography |
| HPLC | High performance liquid chromatography |

Introduction

Dracaena, a genus in the family *Dracaenaceae*, distributed in tropical and sub-tropical areas are composed of about 40 species. Unlike most monocots, dracaenas are evergreen shrubs or trees most frequently characterized by long linear leaves often on unbranched stems or trunks. The plants are cultivated as a tropical foliage plant and propagated for commercial purposes for export (Jones and Luchsinger 1986). The common method of propagation is by using canes or tip cuttings. There is interest to develop methods to induce rapid emergence of adventitious roots. Generation and subsequent growth of adventitious roots in the stem tissues of cuttings and canes is a crucial physiological process in the propagation of many ornamental plant species. Some *Dracaena* sp. may be slow to form adventitious roots and the cuttings disintegrate prior to the formation of functional roots (Rose and Pellett 1994; Stankova and Panetsos 1997; Voyiatzi et al. 2002). Species where roots

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emerge rapidly are often categorized as ‘easy to root’ (Wilkerson et al. 2005) in contrast to those more ‘difficult to root’ ones that require longer periods before emergence of roots (Tarrago et al. 2005).

Despite the intensive control of environmental factors in the modern propagation industry, high economic losses still occur due to insufficient rooting or even decay of the cuttings (De klerk et al. 1999; Druege et al. 2004, 2007; Kadner and Druege 2004). The limited potential to form adventitious may be due to the physiological state of the tissues (Ermel et al. 2000; Reineke et al. 2002). There is a perception that leafy ‘softwood’ cuttings need to form adventitious roots quickly, otherwise tissues will become dysfunctional through either prolonged exposure to sub-optimal environments (Howard and Harrison 1995; Aiello and Graves 1998). In slow to root species, reasons for cutting failure are frequently associated with tissue dehydration (Grange and Loach 1983), loss or an inability to photosynthesize new carbohydrates (Reuveni and Raviv 1981; Del Rio et al. 1991), and possibly a limited response to exogenous auxins (Aminah 2003). Providing environmental conditions that minimize water stress and provide light for photosynthesis appears to be critical (Howard and Harrison 1995). Different attempts to make use of different authentic plant hormones for propagation are well documented. Favorable response was reported in rooting when quick dip in indole acetic acid (IAA) was used in the propagation of tea clones (Kathiravetpillai et al. 1983). Some landscape ornamental plants respond as well to foliar application of auxin as a basal quick dip (Blythe et al. 2004). It is reported that application of IAA and indole butyric acid (IBA) in *Gravillea* showed significant difference in the formation of adventitious roots compared to control (Krisanthini et al. 2006). The mung bean cutting did not show any response to the application of IAA (Jarvis et al. 1985). For those species where success from conventional cuttings remains elusive or inconsistent, tissue culture, grafting or air layering may be employed.

Coconut water (CW) contains growth hormones and is liberally made use in tissue culture. Addition of CW in to the media enhanced the shoot growth and development of medicinal plants propagated in vitro (Tefera and Wannakraioj 2004). Callus initiation of *Catharanthus roseus* have responded favorably when exposed to CW in place of growth hormones in vitro (Namdeo et al. 2006). In vitro experiments have demonstrated that, CW in combination with synthetic auxins such as IBA and naphthelene acetic acid (NAA) can be used for shoot induction and multiplication (Loc et al. 2005).

These methods too, however, do not necessarily guarantee benefits and even when proven useful may have limited application due to cost, the amount of labor

required or low reproduction rates (Saranga and Cameron 2007). Improving propagation methods through the use of conventional canes and cuttings therefore remains a key objective for ornamental plant propagation.

While the isolation of growth factor from CW is reported (Mamaril et al. 1986), their use in the propagation of canes is not documented.

In the present study, hormone extracts isolated from CW was used as a treatment for propagation of the decorative plant variety *Dracaena purplecompacta* L.

Materials and methods

Preparation of raw material

The selected dehusked 6- to 9-months-old coconut fruits were purchased from local market (Kelaniya, Sri Lanka). The fruits were rinsed in water containing 100 mg l⁻¹ of active chlorine (Carvalho et al. 2007). A special stainless steel knife was used to perforate the fruit’s mesocarp. The water of the individual fruits was poured and mixed in a stainless tank. The final volume was filtered using a sterile cotton cloth with the following composition: pH 5.21, 5.1% Brix, 7.92 mS conductivity, 1.8 g l⁻¹ of titrable acidity in HCl, 5.6 g l⁻¹ of reducing sugar and 3.2 g l⁻¹ total free amino acid. Two liters (2.0 l) of CW was used for the extraction.

Coconut water extraction

The CW (2.0 l) was initially set to pH 2.5 with 1.0 M HCl and extracted with ethyl acetate (200 ml × 5). The pH of the aqueous layer was adjusted to 7.0 with 1.0 M NaOH and same extraction procedure was followed at pH 7.0 (Unyayar et al. 1996). The pH of the aqueous layer was adjusted to pH 11.0 with 1.0 M NaOH. The aqueous layer was hydrolyzed at 70°C for 1 h to obtain the bound forms of the hormone in CW. The same extraction procedure was followed as above at pH 7.0 and 2.5, respectively, with ethyl acetate 10% (v/v) to isolate the bound forms of the hormone (Unyayar et al. 1996). The ethyl acetate layer was concentrated under vacuum pressure in rotatory evaporator at 40°C. Concentrated extract was dissolved in 1.0 ml of absolute methanol and stored below -20°C until they were used in the bioassay experiments (Unyayar et al. 1996).

Identification and characterization of plant growth regulator

Chromatography with authentic compounds by thin layer chromatography (TLC) and high performance liquid

chromatography (HPLC) were used to characterize the hormone present in CW. TLC chromatograms were run on 0.50-mm-thick preparative silica gel plates (Silica gel 254 nm, 5 × 20 cm), using chloroform:ethyl acetate:formic acid [50:40:10 (v/v)] to separate IAA (Tien et al. 1979) in the CW extract. The band identified as IAA on the TLC was scraped and redissolved in 1.0 ml of methanol. The IAA extract was filtered through 0.45- μ m filter, 10 μ L was injected into a HPLC reverse phase column Zorbax SD-C18 (4.6 × 25 cm) with particle size 5 μ m, Agilent 1100 series liquid chromatograph equipped with a Agilent 1200 series diode array ultraviolet detector absorbing at 254 nm. Water:acetonitrile:acetic acid [85:15:1 (v/v)] solvent system (Tien et al. 1979) was used to separate IAA in CW extracts. The flow rate was 1.0 ml min⁻¹ and the operating pressure was 113 atm.

Retention times for peaks were compared to those of authentic standards. Quantitation was made by comparison of peak area. Identification of IAA present in CW extract by HPLC is shown in Fig. 1a, b.

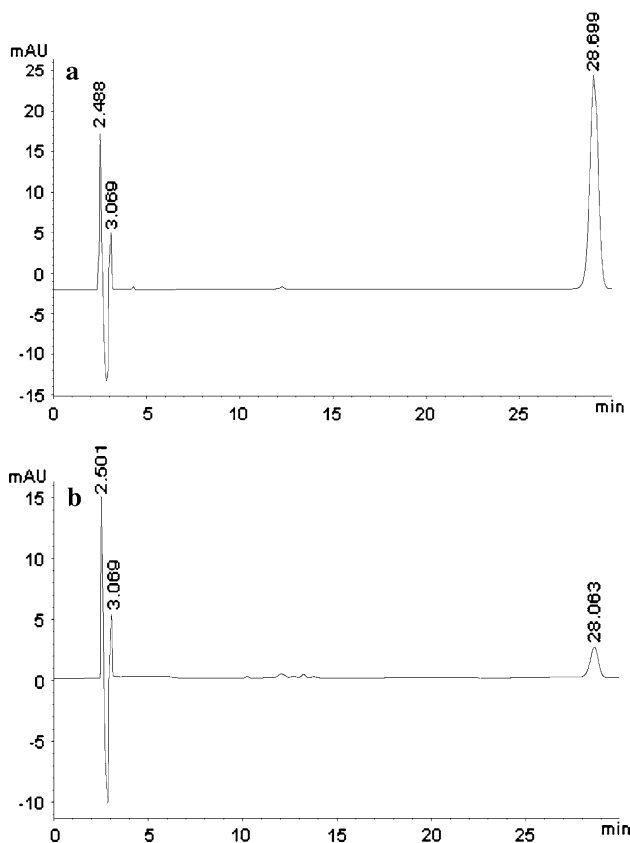


Fig. 1 Identification and characterization of the hormone by HPLC. Authentic compound contained 100 mg L⁻¹ and injection volume was 10 μ L. Chromatogram **a** (top), authentic IAA. Chromatogram **b** (bottom), the separated TLC fraction from the CW extract

Preparation of plant material

Dracaena purplecompacta L. canes were obtained from Serendib Horticulture (Private) Limited. The canes were prepared when stock plants were approximately 1–2 years old. Canes (softwood stem cuttings), approximately 10-cm-long by 5–10 mm wide (caliper) were used in the experiments. The plant material was washed in running water for 5 min. The plant canes were surface sterilized with 95% ethanol and 2.5% sodium hypochlorite, washed three times with distilled water (Tien et al. 1979).

Treatment and vegetative propagation

Concentration series of treatment solutions (28, 57, 143, 286, 571 μ M of natural IAA) were prepared by diluting ethanolic CW extracts in distilled water. The *Dracaena* canes were dipped up to (2.0 cm) in boiling tubes containing 2.0 mL of treatment solution. Another set of treatment was carried out simultaneously with five concentrations (28, 57, 143, 286, 571 μ M) of authentic IAA hormone (MERCK, India). Control canes were dipped in distilled water. Each treatment consisted of 15 canes (five cane triplicates). The tubes containing canes were arranged in randomized block design on an array of racks. The number of roots, total length of roots (mm), number of shoots and number of leaves were recorded per cane basis, at the end of a period of 5 weeks. The environmental conditions, 12 h of normal sunlight, 28–30°C temperature and 70–80% relative humidity were monitored during the 5-week period.

Data collection and statistical analyses

Data were analyzed by ANOVA one way method (MINITAB14.0 software package) to test the significant differences between different treatments at $P < 0.05$ and Tukey's pair-wise comparison test to identify significantly different pairs. Data presented as mean values (Figs. 2, 3).

Results and discussion

The IAA present in CW can be isolated by the method given in the current study (Fig. 1a, b). Reverse phase dialysis may be an option for large scale extraction of hormones from CW. Mature coconut water has been used for the mass propagation of different orchid species like *Dendrobium* sp., *Vanda sanderiana* and *Grandiflora* sp. by tissue culture techniques. (Rethinam and Kumar 2001). While the growth promoters present in coconut water (Marmaril et al. 1986) and milk (Pollard et al. 1961) has been identified, their application to promote rooting has not

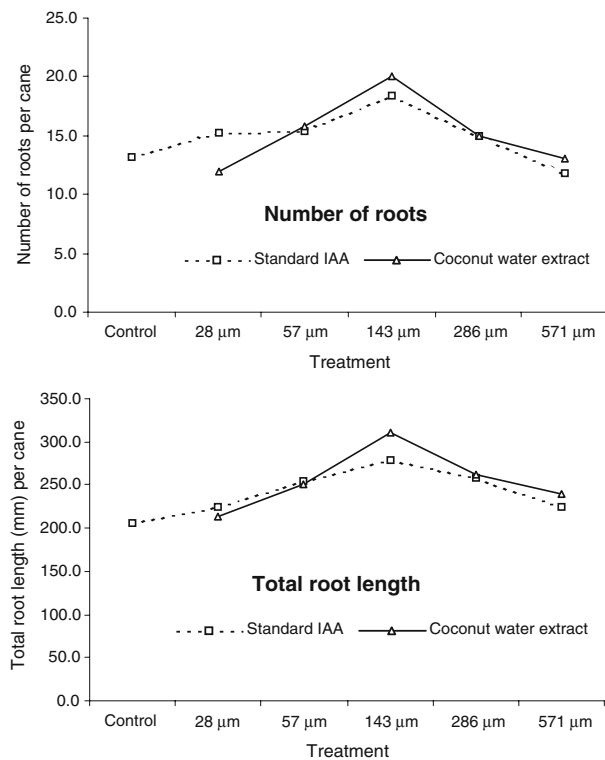


Fig. 2 Rooting response of *Dracaena purplecompacta* L. canes treated with different concentrations of CW extracts and authentic IAA hormone. Each data point represents a mean value of 5 replicates

been explored. Hormones extracted from a natural source may be better than synthetic ones as harmful impurities are absent in such samples.

Canes treated with 143- μ M IAA CW extract exhibited best root induction with 9% more roots and 12% longer roots than 143- μ M authentic IAA treated canes. Compared to the control canes (distilled water) 143- μ M IAA CW extract showed 52% increase in number of roots and also 52% increase in total root length.

Marginally higher number of roots was recorded in the canes, treated with 57- μ M IAA and 286- μ M IAA CW extracts and 28, 57, 286 μ M authentic IAA (Fig. 2). Total root length was higher in all hormone treatments compared to the control canes. The rooting response in *D. purplecompacta* canes show an increase in number of roots and total root length with the increase of concentration up to 143- μ M IAA and a decrease at higher concentrations (Fig. 2).

Shoot generation was also prominent in 143- μ M IAA CW extract treated canes, which showed a 13% increase in the number of shoots compared to the 143- μ M authentic IAA and 100% increase compared to the control canes. With respect to the number of leaves a 16 and 220% increase, respectively, was observed for the 143- μ M authentic IAA treated canes and control canes.

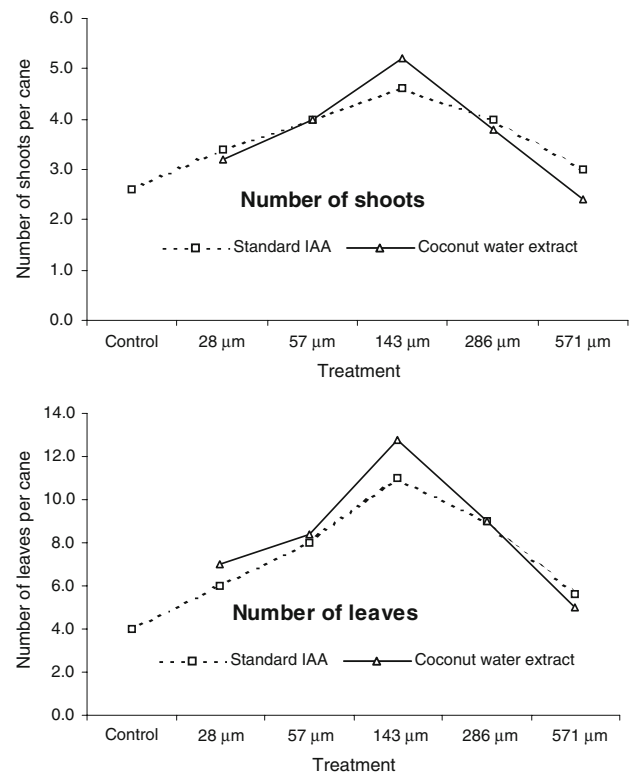


Fig. 3 Shoot generation in *Dracaena purplecompacta* L. canes treated with different concentrations of CW extracts and authentic IAA hormone. Each data point represents a mean value of 5 replicates

Shoot generation in *D. purplecompacta* L. increase in number of shoots and number of leaves with the increase of hormone concentration up to 143 μ M and the response decrease at higher concentrations (Fig. 3).

It is clearly evident in the present study, that IAA CW extract treated *D. purplecompacta* L. canes exhibited better adventitious rooting than those canes treated with similar concentrations of authentic IAA hormone. Low levels of auxins often result in failure of adventitious rooting (Smith and Wareing 1972).

Initiation of lateral roots on the main root is promoted by auxin (Thimann 1977). When oats seeds were germinated on an exogenous auxin solution, the number of roots formed was increased remarkably, but the elongation of the oat seedlings roots was inhibited. (Thimann 1977).

Further, a combination of IAA, gibberellic acid and kinetin increased plant growth when the concentration of IAA and kinetin were very low (0.028 and 0.005 μ M, respectively), and higher concentrations of IAA and kinetin appeared to reduce growth, although differences observed were not statistically significant (Tien et al. 1979). Root hairs and lateral roots were denser when IAA, gibberellic acid, and kinetin were added to the solution. (Tien et al. 1979) The same pattern was observed in the current study;

thus, exogenous application of IAA CW extracts become effective if the endogenous level of the plant growth regulators declined in the *Dracaena* canes. This accounts for rooting response observed for *D. purplecompacta* L. canes treated with plant growth regulators present in CW extract. The present procedure utilizing CW extract is cost effective, and a novel method compared to conventional methods of vegetative propagation of ornamental plants. Our procedure ensures faster and better rooting of *D. purplecompacta* L. propagation in comparison to the conventional method where the canes are stuck in a coir fiber dust bed or some other substrate for longer period of time to achieve the desired level of root and shoot development.

Conclusion

It can be concluded that CW extract containing 143 μM of natural IAA can be used to induce the rooting process of *D. purplecompacta* L. canes. Exogenous application of IAA CW extract is beneficial for induction and growth of adventitious roots in *D. purplecompacta* L. canes for vegetative propagation.

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