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Effect of coconut *(Cocos nucifera)* water extract on the development of adventitious roots in *Polyscias fillicifolia* stem cuttings

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ABSTRACT

Purpose *Vegetative propagation continues to be a popular method to propagate ornamental plant species. Coconut water (CW) can be considered as an abundant source of hormones and other plant growth regulators (PGRs). In this study, vegetative propagation of Polyscias fillicifolia was attempted using PGRs isolated from CW.*

Research method *Stem cuttings were treated with varying concentrations (5, 10, 25, 50,100 µg mL^{-1}) of PGR isolated from CW. The results were compared with a control set (treated with distilled water) and with those treated with solutions of pure synthetic indole acetic acid (IAA).*

Findings *P. fillicifolia canes treated with a 25 µg mL^{-1} concentration of IAA, isolated from CW, showed the highest levels of root induction and development. Root development was more rapid (5 weeks) in the samples treated with PGRs isolated from CW compared to the canes propagated in the field by placing the canes on coir beds in plant nurseries. (6 weeks).*

Originality *This is the first study to use PGRs isolated from CW extracts to improve lateral root proliferation, induce shoot development and leaf emergence in P. fillicifolia.*

Key words: *Plant growth regulators, Polyscias fillicifolia, Coconut water, vegetative propagation*

INTRODUCTION

Polyscias, a genus in the family Araliaceae, consists of approximately 150 species. *Polyscias spp.* are shrubs or trees containing leaves with serrated margins. *Polyscias* are popular as ornamentals and exported from Sri Lanka to various European markets. In Sri Lanka, most varieties of *Polyscias* are propagated from stem cuttings. To facilitate root development, and shoot length, synthetic hormones are usually applied to stem cuttings. However, due to adverse side effects on human health, and the effects on environment, the use of synthetic hormones is not a popular technique (Karunarathna, *et al.*, 2019). Vegetative propagation by means of stem cuttings is widely used in horticulture (Dada *et al;* 2019). In some instances, vegetative propagation is hampered by inadequate rooting, or by high variations in the rooting response (Lee and de Fossard, 1974; Teale *et al.*, 2005). Rapid and uniform development of adventitious roots on stem tissue is an important factor in the propagation of Polyscias spp. for export markets (Druege *et al.*, 2016). Therefore, new methods to induce rapid and uniform adventitious root development in plants are of interest for the Horticulture industry (Teixeira Da Silva, 2003). The application of plant growth regulators (PGRs) is a common practice in horticulture to promote rooting. Indole-butyric acid (IBA), indole acetic acid (IAA), and naptheleneacetic acid (NAA) are commonly used PGRs during the propagation of cuttings. Plant growth regulators generally increase the percentage of rooted cuttings, root initiation rate, and the number of roots per cutting (Amzallag, 1999).

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Application of PGRs to facilitate vegetative propagation has been reported for several plant species. In one instance, a quick dip in a solution of IAA has improved the rooting response in tea (Jayasinghe *et al.*, 2018). Some ornamental plants have responded favorably to applications of auxins to leaves and quick dips of canes (Blythe *et al.*, 2004).

The application of IAA and IBA is considered helpful in adventitious root formation in hard wood plants (Pijut *et al.*,2011). Irrespective of many advances in the methods of propagation, the use of canes and cuttings remains as an important method for ornamental plant propagation with the desired genetic composition. Rapid rooting is an important parameter that ensures successful propagation of stem cuttings. Exogenous synthetic hormones are applied to achieve the desired rate of rooting. The cost of synthetic hormones and the toxicity for human are factors that deter the frequent use of them in plant propagation. Hence, it is important to investigate the effectiveness of PGRs from natural sources in the vegetative propagation of ornamental plants.

Coconut water (CW) is an underutilized by product of the food industry. It is a rich natural source of plant growth regulators such as auxins and cytokinins (Yong *et al.*, 2009). CW also contains kinetin (KIN) that exhibits cytokine-like behavior (Ma *et al.*, 2008). Coconut is reported to have been used in plant tissue culture as a source of zeatin in the *in vitro* culture of olive due to the presence of high levels of zeatin. As zeatin is an expensive compound and replacing it with coconut water makes *in vitro* propagation economically viable. (Souza *et al.*, 2013). Coconut water containing cell culture medium has previously shown to improve the callus growth, shoot regeneration and shoot growth of spinach *(Spinacia oleracea L.)* (Al-Khayri *et al.*, 2019). Hence, CW can be a safe source of phytohormones that can be utilized during vegetative propagation of canes and cuttings. It has also been used as a microbial growth medium. In this study, PGRs isolated from CW were used for the propagation of the decorative plant variety *P. fillicifolia.* The Growth promoting effects of CW extracts have been utilized in plant tissue culture but, such methods do not guarantee benefits due to the high costs and low rates of reproduction (Saranga and Cameron, 2007). Isolation and characterization of growth factors from CW have been reported (Mamaril *et al.*, 1988). Propagation through stem cuttings helps to produce a large number plants with the same genetic composition. *P. fillicifolia* is a popular ornamental plant grown commercially in Sri Lanka. A natural PGRs to enhance growth of cuttings would be economical and environmentally safe. Hence, the aim of this research was to investigate the effect of PGRs isolated from coconut water in the vegetative propagation of woody canes of *P. fillicifolia* .

MATERIALS AND METHODS

Preparation of hormone extracts from coconut water

De-husked, 6-9-month old coconut fruit (n=8) were purchased from Kelaniya, Sri Lanka for the study. Distilled water containing chlorine (100 mg l^{-1}) was used to rinse the fruits (De Carvalho *et al.*, 2007). The fruit mesocarp was perforated with a special stainless steel knife, and the water (CW) from individual fruit was collected and mixed in a cleaned vessel. A sterile cotton cloth was used to filter CW. The filtrate was tested for the following: pH, Brix index, conductivity, titratable acidity, total reducing sugars, and total free amino acids.

To extract the PGRs from CW, method reported by Agampodi and Jayawardena 2009 was used. In brief, the pH of CW was adjusted to 2.5 by adding 1.0 M HCl, then the pH was gradually increased to 7 and then to 11 by adding 1.0 M NaOH. At each pH the aqueous layer was extracted with ethyl acetate. Then the aqueous layer was heated to 70 ^{o}C for 1 h and was further extracted with 10 % (v/v) ethyl acetate to isolate the bound forms of the hormone. The ethyl acetate layers were concentrated under vacuum pressure in a rotatory evaporator at 40 ^{o}C. The resultant extract was stored at -20 ^{o}C until use.

Identification and characterization of PGRs

PGRs in CW extracts were characterized using

chromatography techniques reported earlier (Agampodi and Jayawardena, 2009) . To separate the PGRs, CW extract was placed on preparative thin layer chromatography plates (TLC) coated with silica gel (Merck, Germany). A mixture of chloroform (50 %): ethyl acetate (40 %) : formic acid (10 %) (50: 40: 10 [v/v/v]) was used for the separation (Tien et al. 1979). The isolated IAA band was scraped off and dissolved in methanol (1.0 mL), filtered and 10 µL was injected to a High-performance liquid chromatograph (HPLC, Agilent 1100) coupled to a diode array detector (Tien, Gaskins and Hubbell, 1979). The retention time of the peak was compared with the pure synthetic standards for identification and quantification. The concentrated CW extract contained 65 mg ml^{-1} of IAA after fractionation.

Preparation of plant materials

Canes of *P. filicifolia* were prepared from stock *P. filicifolia* plants that were approximately one to two-years old, obtained from a plant nursery at Kalagedihena, Sri Lanka. Canes horizontally cut into the dimension of 10 cm in length and 5-10 mm in diameter were used in the study. The stem cuttings were cleaned with water and then surface sterilized with a mixture of 95 % (v/v) ethanol and 2.5 % (w/v) sodium hypochlorite, followed by rinsing thrice using distilled water to remove excess sterilizing agents (Tien, *et al.*, 1979).

Propagation of canes

P. filicifolia canes were dipped in 2.0 ml of CW extract solutions containing IAA concentrations equivalent to 5, 10, 25, 50, 100 µg mL^{-1}. Second set was treated with pure synthetic Indole Acetic Acid at the same concentration levels. For the control experiment, a set of canes were treated with distilled water in a similar manner. Glass tubes containing one cane per tube were arranged randomly on racks. The cuttings were arranged out-side the laboratory at the Department of Chemistry, University of Kelaniya under the ambient environmental conditions throughout the five-week period with 12 h of normal day-light, 28-30 ^{o}C temperature and 70-80 % relative humidity. At the end of 05 weeks in each cane, the number of roots, length of roots (mm), number of shoots and the number of leaves were recorded.

Data analysis

One-way ANOVA (MINITAB software package) was used to evaluate the significant differences among treatments at $P<0.05$. Tukey's pairwise comparison test was used to identify significantly different pairs. For each treatment three sets of five replicates were used.

RESULTS AND DISCUSSION

The values obtained for the physiochemical parameters for coconut water i.e. pH, Brix index, conductivity, titratable acidity, total reducing sugars, and total free amino acids were similar to the ones reported in our earlier studies. (Agampodi and Jayawardena, 2007).

P. filicifolia canes treated with 25 µg mL^{-1} equivalents of IAA from a CW extract exhibited the highest level of response for induction of roots with 13% increase ($P<0.05$) in the number of roots and 11% increase ($P<0.05$) in the length of roots compared to 25 µg mL^{-1} pure synthetic IAA treated canes. Compared to control (distilled water treated), 25 µg mL^{-1} CW extract gave a 50% elevation ($P<0.05$) in the total number of roots and a 49% increase ($P<0.05$) in total length of roots as seen in Table 1.

Slightly higher numbers of roots were observed in canes treated with 10 and 50 µg mL^{-1} CW (IAA) extracts or with 10, 50, 100 µg mL^{-1} pure syntheetic IAA (Table 1) compared with the control ($P<0.05$). Total root lengths were greater in all hormone treatments compared with control ($P<0.05$) (Table1). The total length of roots and number of roots, in *P. fillicifolia* canes increased with an increase ($P<0.05$) in the concentration of IAA equivalents of CW extract up to 25 µg mL^{-1}. Thereafter, a decrease ($P<0.05$) in root number and length was observed at higher concentrations (Table 1).

Shoot generation was also higher in canes treated with 25 µg mL^{-1} CW extract, which was 43 % more compared with the control ($P<0.05$). A 4.5% increase ($P<0.05$) in the number of leaves were observed in comparison to the control (Table 2).

Table 1. Root induction in *Polyscias fillicifolia* canes treated with CW extracts and pure synthetic IAA hormone analyzed after 5 weeks of propagation.

Treatment		Number of roots (±SD)	Total root length (mm) (±SD)
Control		6.0 ± 0.31 [a]	341 ± 17 [a]
Pure Synthetic IAA	5 μg mL^{-1}	6.4 ± 0.32 [a]	348 ± 17 [a]
	10 μg mL^{-1}	7.0 ± 0.35 [a]	400 ± 20 [a]
	25 μg mL^{-1}	8.0 ± 0.41 [b]	460 ± 23 [b]
	50 μg mL^{-1}	7.2 ± 0.36 [a]	398 ± 20 [a]
	100 μg mL^{-1}	7.0 ± 0.35 [a]	380 ± 19 [a]
CW extract	5 μg mL^{-1}	6.7 ± 0.34 [b]	354 ± 18 [a]
	10 μg mL^{-1}	7.4 ± 0.37 [b]	415 ± 21 [a]
	25 μg mL^{-1}	9.0 ± 0.45 [b]	509 ± 25 [b]
	50 μg mL^{-1}	7.5 ± 0.38 [b]	410 ± 21 [a]
	100 μg mL^{-1}	6.7 ± 0.34 [b]	398 ± 20 [a]

[1]*CW - Coconut water, IAA – Indole acetic acid*

[1]*Different letters denote the significant difference between the treatments (P<0.05) ANOVA one way and Tukey's multiple comparison tests. Data represent the mean value of fifteen replicates.*

Table 2. Shoot generation in *Polyscias fillicifolia* canes treated with CW extracts and pure synthetic IAA hormone analyzed after 5 weeks of propagation.

Treatment		Number of shoots (±SD)	Number of leaves (±SD)
Control		1.4 ± 0.07 [a]	4.4 ± 0.22 [a]
Pure Synthetic IAA	5 μg mL^{-1}	1.4 ± 0.07 [a]	4.4 ± 0.22 [a]
	10 μg mL^{-1}	1.6 ± 0.08 [a]	4.4 ± 0.22 [a]
	25 μg mL^{-1}	2.0 ± 0.11 [b]	4.6 ± 0.23 [a]
	50 μg mL^{-1}	2.0 ± 0.11 [b]	4.6 ± 0.23 [a]
	100 μg mL^{-1}	1.6 ± 0.08 [a]	4.4 ± 0.22 [a]
CW extract	5 μg mL^{-1}	1.6 ± 0.08 [a]	4.4 ± 0.22 [a]
	10 μg mL^{-1}	1.6 ± 0.08 [a]	4.4 ± 0.22 [a]
	25 μg mL^{-1}	2.0 ± 0.11 [b]	4.6 ± 0.23 [a]
	50 μg mL^{-1}	2.0 ± 0.11 [b]	4.6 ± 0.23 [a]
	100 μg mL^{-1}	1.3 ± 0.07 [a]	4.4 ± 0.22 [a]

[2]*CW - Coconut water, IAA – Indole acetic acid*

[2]*Different letters denote the significant difference between the treatments (P<0.05) ANOVA one way and Tukey's multiple comparison tests. Data represent the mean value of 15 replicates.*

IAA equivalent of CW concentration up to 25 μg mL^{-1} gave an increase ($P<0.05$) in the number of shoots and leaves for *P. fillicifolia* canes. However, the response decreased ($P<0.05$) beyond 50 μg mL^{-1} (Table 2). In this study, treatment with CW extract or with IAA up to 25 μg mL^{-1} improved rooting, shooting, and leaf generation in *P. fillicifolia.*

Teale *et al.*, 2005 reported that low concentrations of auxins often lead to the inhibition of adventitious root development. However, lateral roots development on the main root was facilitated by auxin (Cline, 1996). In his study, Cline showed that root elongation in seedlings was inhibited by auxins, but when the seeds were treated with an exogenous solution of auxin, the number of roots formed increased. Treatment with a combination of very low concentrations of IAA (0.005 mg L^{-1}) and KIN 0.001 mg L^{-1} along with Gibberellic acid increased the plant growth. However, when the concentration of IAA and kinetin was increased, the growth was reduced but not in a statistically significant manner (Tien, *et al.*, 1979).

Exogenous application of a CW extract becomes more significant if the endogenous level of the PGRs decline in the *Polyscias* canes. This may account for the high rooting response of *Polyscias* canes due to PGRs present in the CW extract. Coconut water is a natural source of hormones. Hence, PGRs extracted will be free of any harmful impurities compared to pure synthetic hormones. Therefore, the natural combination of PGRs may have a beneficial effect compared to the application of individual pure hormones.

The protocol reported in this study utilizing CW extract is an environmentally friendly, cost effective method that could be recommended for vegetative propagation of ornamental plants. The current procedure ensured rapid (5 weeks) and better rooting of *P. fillicifolia* canes than the method (6 weeks) used in the horticulture industry. Popular method used in *P. filicifoilia* propagation is to place the canes in coir fiber dust beds. The duration to reach the desired level of root development and shoot elongation is about six weeks. Hence, use of a hormone extract from CW for vegetative propagation of *P. fillicifolia* canes could be recommended as a safe and quicker alternative which could be utilized in horticulture industry especially for rooted cane export purposes.

CONCLUSION

CW extract (25 μg mL^{-1}) induced the root development of *Polyscias fillicifolia* canes. Application of CW extract can be recommended for rapid induction and growth of lateral roots in *Polyscias fillicifolia.*

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