Cey. J. Sci. (Bio. Sci.) 39 (1): 45-51, 2010

ROOT GROWTH PROMOTION OF *FICUS* SPECIES DURING AIR-LAYERING

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Accepted 30 May 2010

ABSTRACT

Three chitosan concentrations (1%, 3%, 5% w/v) were tested for their root growth promotion properties in air-layering of three popular ornamental *Ficus* species namely *Ficus triangularis*, *Ficus microcarpa* and *Ficus benjamina*. Subsequently, these treatment responses were compared with a rooting hormone (naphthalic acetic acid) and an organic root promoting extract (Root Most) available in the local market. Tap water was used as the control. Air-layered plants were further subjected to export simulation for 14 days at 12-14 ^{0}C and 90% relative humidity (RH) with the aim of lengthening the storage period. 3% (w/v) chitosan treatment concentration was shown to promote the root growth significantly. 3% (w/v) chitosan treatment resulted in the highest mean root length of (15.3 cm) in *F. triangularis* compared to the 10.3 cm of tap water control. Similarly, highest mean root length of 38.8 cm was recorded for *F. microcarpa* for 3% (w/v) chitosan treated layers compared to 14.8 cm of tap water treated layers. Further, the highest mean root density of 3.17 was recorded for 3% (w/v) chitosan treated layers of *F. triangularis.* A cost effective packaging system was developed during this research which could be utilized in cold storage of air-layers during air or sea freight.

Key words: 3% (w/v) chitosan, packaging, cold storage, export

INTRODUCTION

Plants of genus *Ficus* are widely used as ornamental indoor and outdoor plants all over the world. Shiny beautifully shaped leaves and flexible trunks increase their popularity. *Ficus benjamina*, *Ficus microcarpa* and *Ficus triangularis* are three very popular ornamental species widely used in indoor and outdoor environments. All these *Ficus* varieties are favorably grown in tropical climates and do not require extensive care or special fertilizers. Therefore, these can be easily grown in Sri Lanka in larger quantities. As they have a high export potential, they can be cultivated commercially for exportation thereby earning foreign exchange (Jiffry, Personal communication).

Rooting of cuttings, air-layering, and tissue culture are primary methods of *Ficus* propagation (Acquaah, 2002; Rice and Rice, 2000; Crockett, 1972). Due to the difficulty and cost factor involved in rooting using tissue culture, air-layering is considered as the best method for *Ficus* propagation. Air-layering and application of rooting compounds is practiced in most commercial environments of plant propagation (Acquaah, 2002; Rice and Rice, 2000; Crockett, 1972).

To achieve maximum profit levels in commercial environments, alternative rooting compounds having better qualities should be developed at a lower cost. Chitosan is such a product which is produced from the waste accumulated in the shell fish processing industry (Hien, 2004). Chitosan is reported to possess antimicrobial properties especially antifungal properties while promoting the growth of plants (Hien, 2004). Low molecular weight oligosaccharides of chitin can be produced by irradiation degradation of chitosan. Oligochitosan has been recognized as a potent phytoalexin inducer as well as a growth promoter of plants (Hien, 2004).

In this research identified chitosan treatment concentrations were tested along with an organic rooting treatment and commercially popular rooting hormone (Naphthalic acetic acid: NAA) to identify and compare their root growth promoting activities. Thereby, the potential of applying chitosan in commercial air layering

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practices was identified. Subsequently, an export stimulation study was conducted under cold storage using a cost effective packaging system. Finally stored plants were re-planted to assess the establishment potential of those plants.

MATERIALS AND METHODS

Plant materials

All experiments were conducted using plants of three *Ficus* species; *F. benjamina*, *F. microcarpa* and *F. triangularis.* Experiments were carried out during the morning hours (between 9.00 am- 11.00 am), at the botanical garden of the Department of Botany, University of Kelaniya, Sri Lanka.

Preparations of treatments

1, 3 and 5% (w/v) chitosan treatments were prepared by dissolving chitosan flakes (Nuclear Research Institute, Vietnam) in 1% acetic acid (Norfork, U. K.). pH of these solutions were adjusted to around 6.0 using 1N sodium hydroxide (BDH Limited England) with the aid of a pH meter (HI98107, Portugal) (Hien, 2004).

One ml of the organic rooting compound (Root Most, LEILI Agrochemistry Co. Ltd, Beijing, China) was dissolved in 99 ml of distilled water to prepare the organic rooting treatment.

Rooting hormone (1- napthylacetic acid and thiram) (Secto, Sinclair Animal and Household Care Ltd., Gains Borough) was also prepared by dissolving 1g of rooting hormone powder in 5 ml of distilled water obtaining a paste. Tap water was used as the control.

Selection of branches

Young, healthy, vigorously growing branches of same maturity stage having the diameter of about 1 cm were selected from the *Ficus* plants at the botanical garden of the Department of Botany, University of Kelaniya.

Air-layering

Branch wounding and application of treatments

All the leaves and twigs on the selected branches were removed above and below the point where the cut was made (55 cm below the shoot tip). Branches were injured by removing a one inch ring of the bark and the cambium layer by making two parallel cuts and by joining those cuts with a single transverse cut (Acquaah, 2002).

Each wound was treated with the selected treatments. Each treatment was directly applied on the wound using a paint brush. Paint brushes of the same size were used for the application of all the treatments. About 2 ml of each treatment was directly applied on the wound.

Treatments applied were 1, 3 and 5% (w/v) chitosan, organic root promoting extract solution, rooting hormone and tap water (control). Each treatment was replicated six times (Acquaah, 2002).

Enclosing the wounds

Treated wound sites were enclosed with moist coir dust and wrapped with low density polyethylene (150 gauge). Layers were tightly secured with polythene by cotton strings. Afterwards each layer was enclosed with aluminum foil to reflect the excess light that reaches the layer thereby preventing the build up of excessive heat inside the layer. Prepared layers were allowed to root and were regularly observed for root growth (Acquaah, 2002).

Assessing air-layers

Number of days required for the root emergence and the number of days required for drying of the coir dust medium was taken in to consideration in each *Ficus* species, and periods were determined to remove air- layers (*F. benjamina* - 21 days, *F. triangularis* – 22 days, *F. microcarpa* – 60 days). The layers were removed from the mother plant just below the wounded area according to the above periods identified (Acquaah, 2002).

Air-layers were assessed after removing the medium and careful washing with tap water without damaging the root system. Parameters used to assess plants were: lengths of the six longest adventitious roots, number of adventitious roots per plant and root density (lateral branching of the adventitious root system).

Density of the roots were assessed using a numerical index of 1-5, developed at the department of Botany, University of Kelaniya (1 = No branching, 2 = Few branches, 3 = Normally branched, 4 = Densely branched, 5 = Very densely branched).

Cold storage/ Export simulation

After assessing the layers they were placed and sealed in low density polyethylene bags after enclosing the root system in wet cotton wool. Subsequently the bags were packed in telescopic type cardboard boxes (45×30×30 cm). Ten plants were packed in each box. The boxes were placed in a cold room for one to two weeks at 85 – 90% relative humidity at 12–14 ^{0}C.

Establishment of plants in soil after storage

At the end of the storage period boxes were taken out from the cold room and all the packing materials were removed. Subsequently, the layers were brought in to normal atmospheric temperature.

Air-layers were then planted in high density polyethylene bags (8 ×10 inches). Soil mixture was prepared by mixing equal proportions of compost and sand. Plants were watered depending on the requirement and kept under shade. Survival of the plants was assessed after 45 days and the survival percentage of each plant species was calculated.

Statistical analysis

The data obtained for parameters: lengths of the six longest roots, number of roots per plant, were analyzed using one-way ANOVA parametric analysis.

Lateral branching (density of the root system) of the root system was analyzed using Kruskal-Wallis non parametric test. Scores were assigned to root systems according to their density. Statistical analysis was done using the Minitab version 14.

RESULTS

Mean number of roots emerged per plant in air-layered Ficus species

According to the statistical analysis, most treatments did not show a significant root number increment. However, 3% chitosan treatment showed marked root increment in the case of *F. triangularis* air layers (>39) compared to the other treatments (<35). However, the observed difference was not significant.

Length increment of six longest roots of air-layered Ficus species

Ficus triangularis

The root growth promoting effect of certain treatments was evident by higher root lengths. Mean length of six longest roots per air-layered, *F. triangularis* was significantly different in tap water control (10.3 cm) and 3% chitosan (15.3 cm) treatments. As shown in Figure 1, highest mean root length was observed in 3% chitosan treatment followed by 1% chitosan and organic rooting treatment. Tap water treated plants showed the minimum number of roots.

Ficus microcarpa

Mean length of six longest roots per air-layered *F. microcarpa* using treatments such as organic rooting treatment, 1% chitosan, after 60 days was not significantly different according to the statistical analysis. However, mean root lengths were significantly different to other treatments, in 3% chitosan (24.9 cm) and tap water (14.8 cm). Mean root length of 3% chitosan was also significantly different compared to 5% chitosan (16.8 cm) and naphthalic acetic acid (17.8 cm) treatments. The significant positive effect shown by 3% chitosan on root length is clearly illustrated in Figure 2.

Ficus benjamina

None of the treatments showed any significantly different effects on the root systems of *Ficus benjamina* air-layers.

Root density of air-layered Ficus plants before export stimulation

Ficus triangularis

According to the Kruskal-wallis non-parametric statistical analysis, root density of the layers treated with the organic rooting treatment, 3% chitosan and 5% chitosan were significantly different and higher than the root density of the layers treated with tap water control. Root density of the layers treated with 1% chitosan and naphthalic acetic acid were not significantly different from tap water.

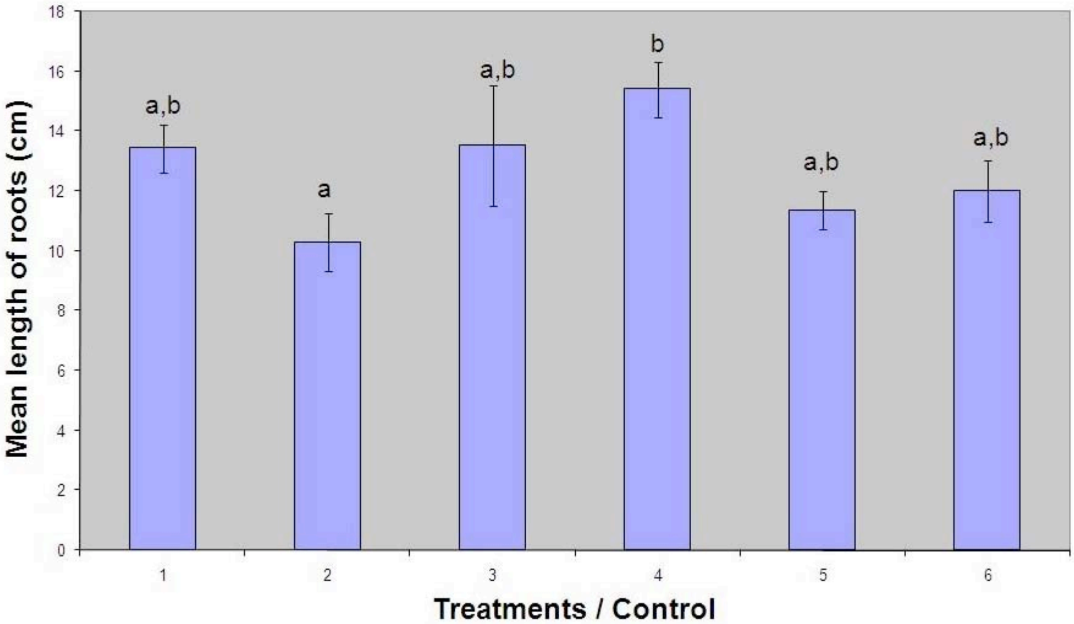

Figure 1. Mean length of six longest roots per plant treated with 1 - organic rooting compound, 2 - tap water, 3 - 1% chitosan, 4 - 3% chitosan, 5 - 5% chitosan and 6 - naphthalic acetic acid on air-layered *Ficus triangularis* plants after air layered branches were removed from the mother plant (after 22 days).

Each data point represents the mean of six replicates ± standard error. Means followed by the same letters are not significantly different at p=0.051.

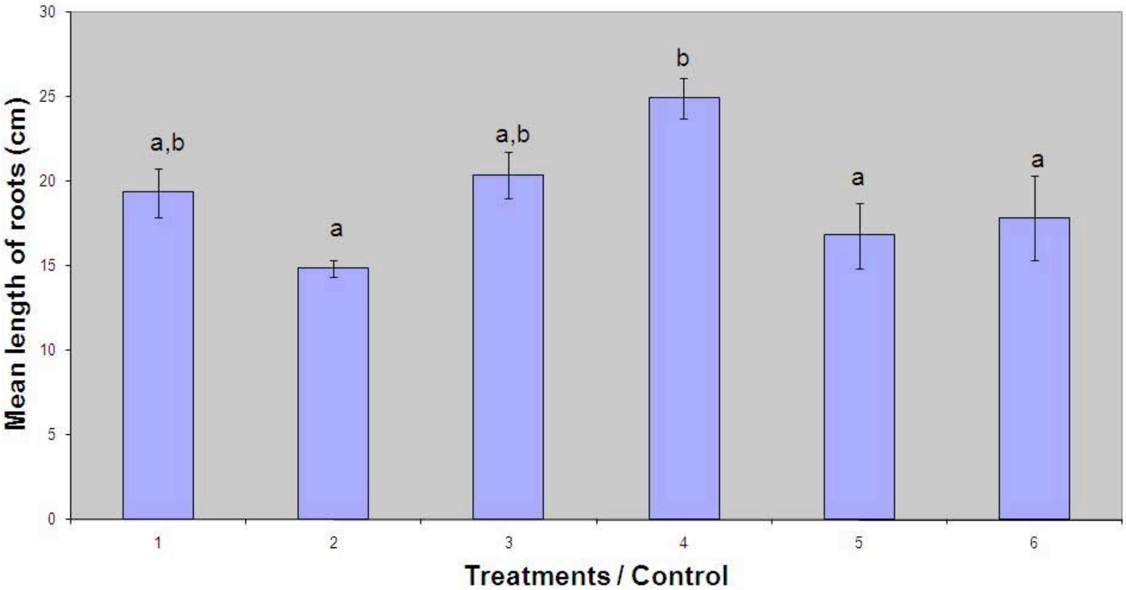

Figure 2. Mean length of six longest roots per plant treated with an 1 - organic rooting compound, 2 - tap water, 3 - 1% chitosan, 4 - 3% chitosan, 5 - 5% chitosan and 6 - naphthalic acetic acid on air-layered *Ficus microcarpa* plants after air layered branches were removed from the mother plant (after 60 days).

Each data point represents the mean of six replicates ± standard error. Means followed by the same letters are not significantly different at p=0.002.

Ficus microcarpa

Root density of the plants treated with an organic rooting treatment, 1% chitosan and naphthalic acetic acid were significantly different and low compared to the root density of the plants treated with tap water control. Root density of the plants treated with 3% chitosan and 5% chitosan were not significantly different than that of the tap water treatment.

Ficus benjamina

When root density of *Ficus benjamina* was taken in to consideration, significantly different effects could not be observed with regard to the treatments.

Establishment of plants in soil after storage

The survival percentage of established air-layers after subjecting them to export simulation was not markedly different with regard to the treatments. Air-layers of all three *Ficus* spp. showed satisfactory establishment potentials (survival percentages > 60%) after subjecting them to cold conditions.

DISCUSSION

Ficus benjamina, *Ficus triangularis*, and *Ficus microcarpa* are three most popular *Ficus* species of the family Moraceae which have a decorative value in indoor and outdoor environments (Crockett, 1972).

Main method of *Ficus* propagation is air-layering (Acquaah, 2002; Rice and Rice, 2000; Crockett, 1972). Exportation of air-layered plants via air freight and sea freight takes 2-3 days or 2 weeks, respectively. After harvesting layers, they are kept at room temperature until they are exported under cold conditions. If cold storage conditions are applied immediately after harvest, until they are exported, shelf life could be significantly improved. This will result in fewer post harvest loss of air-layered plants (Jiffry, Personal communication). Therefore, in this study an export simulation was conducted with the aim of identifying (1) survival percentage of air-layers and (2) establishment potential of layers.

During this research, root growth promotion property possessed by different treatments was investigated. Although, the number of roots emerged per plant was not significantly different with regard to the treatments, in the case of *F. triangularis,* markedly higher number of roots was obtained for 3% (w/v) chitosan treated plants. Considering the mean root lengths, 3% (w/v) chitosan treatment exhibited a significantly positive effect in *F. triangularis* and *F. microcarpa* air-layers. The other treatments also resulted in a higher number of roots compared to tap water. Chitosan when used at 3% (w/v) concentration proved to possess strong root growth promotion properties compared to the other treatments in the case of *F. triangularis* and *F. microcarpa* air-layering.

In air-layering, plants tend to put more energy on root growth than on shoot growth (Rice and Rice, 2000). Therefore, root growth was intensively observed in almost all the experiments performed. Three percent (w/v) chitosan treatment exhibited better performance in root growth promotion compared to organic rooting treatment and rooting hormone (NAA). Chitosan triggers the defensive mechanisms in plants, stimulates root growth and induces certain enzymes such as chitinases, pectinases and glucanases (Hien, 2004). Due to all these reasons chitosan may have performed better than the commercial rooting products.

The positive impact of chitosan on root growth has been reported in previous studies. For example, supplementation of chitosan to plantlet cultures resulted in a significant increase in the root length for three plant species: *Chrysanthemum*, *Lisianthum* and strawberry (Luan *et al.*, 2005). In another instance, chitosan when introduced in to the medium of orchid tissue culture, was found to possess a profound effect on growth and development of root tissues (Nqe *et al.*, 2005). Not only direct chitosan application, but also biological preparations with chitosan were found to enhance the root growth in some instances. The application of commercial chitosan-based plant growth promoting rhizobacteria as soil amendment in rice fields resulted in two fold increase in shoot and root length and grain yield (Vasudevan *et al.*, 2002).

Lateral branching of roots was expressed as ‘root density’. Accordingly, in *Ficus triangularis* layers, root density of plants treated with an organic root promoting extract, 3% chitosan and 5% chitosan were significantly different and higher than that of tap water control (Table 1). In a previous study it was found that, Indole acetic acid at 2 mgl^{-1} concentration significantly increased root lengths and the frequency of lateral roots in root clones of *Rehmannia glutinosa* (Hwang, 2005). Organic rooting treatment was prepared using a see weed extract. Seaweed extracts contain all major and minor plant nutrients, many trace elements, alginic acid, auxins, gibberellins and antibiotics. Alginic acid is a soil conditioner which, enhances the soil structure for better performance of plants. The other constituents of sea weed extracts promote plant growth (http://www.journeytoforever.org/farm_library/seaweed.html, 2009/11/13). The reason behind the better root growth promotion property exhibited by this treatment could be due to the presence of above elements in the extract.

However, considering the overall result for the growth of the root systems, different chitosan treatments showed root growth promotion properties equal to or better than commercial rooting treatment. Especially, 3% chitosan treatment showed a significant positive impact on the root system followed by 1% chitosan treatment concentration. This clearly proves the positive root growth promotion property associated with 3% and 1% chitosan treatments when they are used as wound treatments in air-layering of *Ficus triangularis* and *Ficus microcarpa* plants. Five percent chitosan treatment however, proved to possess deleterious effects in most instances. The root systems treated with 5% chitosan exhibited reduced performances than the root systems treated with tap water control resulting in lesser root lengths, lesser root numbers and less lateral branching. Therefore, chitosan concentrations equal or higher than 5% may adversely affect the root systems of the three *Ficus* species tested.

After removing the layers from the mother plant, they were subjected to export simulation and established on soil to assess the survival percentage. Although, none of the treatments have shown significant positive impact on the survival of re-planted air-layers, the survival percentages were satisfactory (> 60) with regard to all three *Ficus* species tested. Five percent chitosan treatment concentration exhibited lower survival percentages (<60) of re-planted air-layers compared to other chitosan treatment concentrations and tap water.

In a previous research of tissue culture on *Chrysanthemum*, *Lisianthus* and strawberry, supplementation of culture medium with chitosan was found to improve the survival ratio of the transferred plantlets under green house conditions (Luan *et al.*, 2005). Borkowsky *et al.*, 2007 reported the increased vigour of tomato plants due to chitosan application. During the present research it was difficult to identify any significant positive effects of chitosan on the survival of re-planted air-layers. This also could be due to the removal of the medium around the chitosan coated wound. Disturbances caused to the root system during the removal of medium could also have adversely affected the survival of re-planted layers (Borkowsky *et al.*, 2007). However, a previous research carried out using seed priming with chitosan under low temperature stress reported, enhanced germination index, increased shoot height, root length and shoot and root dry weight in maize plants compared to that of the control (Guan *et al.*, 2009).

Thus, a concentration of 3% (w/v) chitosan could be used as a wound treatment in air-layering of *F. microcarpa* and *F. triangularis* as it provides anti-microbial protection while promoting the growth of plants. Approximately a cost of only 68 cents per one air-layer would be needed for the treatment. When the cost of packaging material is taken in to account, the cost of chitosan treatment for a cardboard carton with ten air-layers would be approximately Rs. 50.00.

Table 1. Root density of air layered *Ficus* plants.

Treatment	Mean density of roots (before export stimulation)	
	Ficus triangularis	*Ficus microcarpa*
Organic root promoting extract	3.1 ± 0.3 [a]	2.7 ± 0.2 [a]
Tap water	2.5 ± 0.6 [b]	3.3 ± 0.2 [b]
1% chitosan	2.7 ± 0.2 [b]	2.6 ± 0.3 [a]
3% chitosan	3.2 ± 0.4 [a]	3.0 ± 0.4 [b]
5% chitosan	2.8 ± 0.6 [a]	3.2 ± 0.4 [b]
Naphthalic acetic acid	2.0 ± 0.4 [b]	2.2 ± 0.4 [a]

Each data point represents the mean of six replicates ± standard error
Means followed by common letter(s) are not significantly different ($p > 0.05$) by Kruskal-wallis non-parametric analysis

CONCLUSION

According to the present research findings, 3% (w/v) chitosan could be recommended to treat the wounds in air-layering of *F. triangularis* and *F. microcarpa*. The cost effective packaging system developed, can be utilized when exporting the air-layers under cold conditions via air or sea freight.

ACKNOWLEDGEMENTS

We gratefully acknowledge the technical assistance provided by staff members of the Department of Botany, Mr. Padmasiri Perera, trained gardner, botanical garden, University of Kelaniya, Sri Lanka and Mr.Faiz Jiffry of Serendib Horticulture Pvt. Ltd., Sri Lanka.

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