



Production of High Quality Air-layers from *Conocarpus erectus* L. in Response to IBA and *Bacillus subtilis*



Essam Y. Abdul-Hafeez

Department of Ornamental Plants and Landscape Gardening, Faculty of Agriculture, Assiut University, Assiut, Egypt.

THE CURRENT study was conducted to evaluate the rooting behavior of air-layers of Buttonwood (*Conocarpus erectus* L.) under IBA stimulation and/or inoculation with *Bacillus subtilis*. Experiment contained two branch lengths (275 and 150 cm) and six treatments of IBA (100, or 200 ppm) and *B. subtilis* suspension (5 or 10 ml/air-layer from suspension 10^8 CFU/ml) added individually or in combinations. The best results were obtained by 200 ppm IBA and/or 10 ml *B. subtilis* resulting in the maximum rooting percentage (100%), the highest number of roots (58.66/layer), the longest root (26.50 cm), large root volume (86.46 cm³/layer), the heaviest fresh and dry roots (82.68 and 13.50 g/layer, respectively) and maximum percentage of layers showing secondary roots (100%). These results were correlated with highest total carbohydrates content (28.40%) and total phenolic compounds (52.41 mg Gallic acid equivalent/g DW). The interaction effects indicated that the superior combined treatment was occurred by shoot length of 150 cm supplied with 200 ppm IBA + *B. subtilis*.

Keywords: *Conocarpus erectus*, Air-layering, Asexual propagation, IBA, *Bacillus subtilis*.

Introduction

Buttonwood (*Conocarpus erectus* L.), an evergreen tree species of the family Combretaceae, has been found to tolerate extreme desert heat where summer temperature may reach 47 °C and to grow in low fertile soils (Branney, 1989). This species is characterized by fairly rapid growth, endurance tropical sun and surviving high salinity levels. It has several uses including providing food and cover for wildlife, soil protection during rainstorms and fixing dunes against migration (Popp et al., 1989). It is widely planted as an ornamental evergreen tree in yards, parking lots, streets and parks as well as a bonsai potted plant. The wood durable and is used to make railroad ties, posts, boats, fuel and charcoal. In addition, its bark and leaves have been employed in tanneries and traditional medicine (Gliman and Watson, 1993).

The application of air layering in plant propagation dates back to 20 centuries ago when Chinese used it for the first time and ever since, it has been known to horticulturists as a method of propagating ornamental and cultivated plants (Mergen, 1953). Vegetative propagation through air layering has an advantage over other methods, since reserve food of the parent branch and the maintained supply of water and nutrients induce the formation of a well-developed root system. Comparing to the root system of cuttings, the air-layered branches develop more balanced and rapid root system. Therefore, air layering is the best option for multiplication of forest tree species whenever, the formation of roots from cuttings is slow (Hartmann et al., 2010).

Various classes of growth regulators, such as the auxins, cytokinins, gibberellins, ethylene and

inhibitors like abscisic acid influence root initiation (Cambell et al., 1999). Many previous studies have demonstrated the optimum concentration of rooting hormones for cuttings and air-layers in different woody plants. The main purpose for applying root promoting substances in layering is to induce high rooting percentage within a short span of time (Bose et al., 1997). Indole-3-butyric acid (IBA), as a synthetic auxin, is commercially famous as a root-initiation promoter (Waisel et al., 1991) since it is more stable and shows flexibility regarding the range of applied concentration (Audus, 1972).

In many cases, bacteria that colonize plant roots are useful for plant growth, development and productivity (Arshad and Frankenberger, 1998). In addition, the synthesis of plant hormones such as auxins, gibberellins and cytokines by microorganisms is believed as one of the main forms of interactions between the plant and the microorganisms which have been proved in various types of bacteria. For example, Khalid et al. (2004) revealed that many rhizosphere bacteria including *Streptomyces*, *Bacillus* sp and *Azotobater* sp. are able to synthesize IAA as secondary metabolites. Indole acetic acid produced by the bacteria works in conjunction with the internal plant auxin to stimulate root proliferation, division of cells and nutrient uptake from the culture media (Leveau & Lindow, 2005 and Khamna et al., 2010).

Hence, the present investigation was conducted to study the effect of branch length and the application of IBA and/or *Bacillus subtilis* on air layering of *Conocarpus erectus*.

Materials and Methods

The current investigation was carried out at the Floriculture Farm, Faculty of Agriculture, Assiut University, Egypt, during the 2017 and 2018 seasons to determine the effects of branch length and the application of indole-3-butyric acid (IBA) and/or a beneficial microorganism (*Bacillus subtilis*) on rooting and survival percentages, as well as root characteristics of *Conocarpus erectus* air-layers (the trees are eight years old).

Active strain of *Bacillus subtilis* was obtained from the Unit of Biofertilizers, Fac. Agric., Ain

Shams Univ., Egypt. Bacterial suspension of *B. subtilis* (10^8 CFU/ml) was supplied to rooting substrate after girdling process of *C. erectus* shoots.

Two lengths of air-layered branches were 275 and 150 cm, were treated with IBA solution individually or mixed in combinations with *B. subtilis* suspension with rooting medium. The treatments included control (untreated), 10 ml IBA (200 ppm), 5 ml *B. subtilis*, 10 ml *B. subtilis*, 10 ml IBA + 5 ml *B. subtilis*, 10 ml IBA + 10 ml *B. subtilis*.

In mid-March of both seasons, selected mature shoots of *C. erectus* (70 trees were used for the study, from each tree used 2-3 branches) were girdled by removing 2.0 cm ring of bark in the intermodal region, treated with the different treatments and the girdling region was immediately covered with a rooting substrate (perlite and peat moss "1:1 v/v"). The rooting substrate was covered with transparent plastic bags and the both ends were secured firmly using gunny thread and then covered with aluminum foil. Only distilled water was used for treating air-layers in the control treatment.

The experiment was arranged in a complete randomized block design in a split plot; the main plot was the two branch lengths (275 and 150 cm) and the sub-plot included the six treatments. Each treatment included 9 layers and replicated three times. The air-layers were protected from any mechanical damage and loss of moisture.

Ten-weeks after, all the air-layers were detached from mother plants, then dipped in water to remove adhering rooting substrate. Data were recorded on rooting percentage, and root parameters. Roots were washed carefully under running water and root portion of each air-layer was separated. The washed root system was wiped with blotting paper to remove additional free water and then root volume was determined by volume-displacement techniques involving suspending roots in a clear graduated glass cylinder to observe the volume displacement (Harrington et al., 1994). Samples of the basal 2.5-3.0 cm portion of root zone for determining total carbohydrates and phenols were oven-dried

at 70° C for 48 h and ground to a fine powder. Total carbohydrates content was determined colorimetrically using anthrone sulphuric acid method described by Fales (1951). The total phenolic content was estimated colorimetrically by Folin-ciocalteau reagent (FCR) method (Maliauskas et al., 2004). After detaching the rooted layers from mother plants, they were planted in polyethylene bags filled with clay soil and were kept under plastic house conditions (3 layers were used for chemical analysis and 6 layers were transplanted). Three months later, the survival percentage was recorded.

Data obtained were statistically analyzed using Statistix 8.1 analytical software and the means were compared using a least significant difference (L.S.D.) test based on Gomez and Gomez (1984).

Results and Discussion

The results obtained during the present experiment clearly showed that the main and interaction effects of air-layered branch length and IBA and/or the beneficial microorganism *Bacillus subtilis* have significant effects on rooting behavior of *Conocarpus erectus* air-layering except on the rooting and survival percentage of planted layers which showed no significant differences as presented in Fig.1.

Rooting percentage and root characteristics

Data presented in Table 1 show non-significant effects of both investigated factors (length of air-layered branch, IBA and/or *B. subtilis*) during both seasons.

Results presented in Tables 2-6 indicate that there are significant differences among all treatments comparing with control and between them under both factors and their interactions in relation to rooting percentage and root (number, length, volume, and fresh and dry weights) characteristics. As shown in table 2, the highest root number was achieved in the shorter air-layers (150 cm). Air-layers treated with 200 ppm IBA + *B. subtilis* (10 ml) showed superiority in comparison with other application treatments during both seasons, the best interaction was in the longer air-layers (275 cm) which treated with 200 ppm IBA and *B. subtilis* (10 ml). The same results were observed regarding root length, Table

3, where the longer roots were observed in the shorter air-layers (39.39 and 41.69 cm) in both seasons, respectively. Concerning the effect of IBA and *B. subtilis*, the longest root, respectively was observed in the air-layers treated with *B. subtilis* (5 ml) during both seasons (23.33 and 24.74 cm). The shorter air-layers treated with IBA (200 ppm) and *B. subtilis* (5 ml) gave the longest roots (25 and 26.5 cm) in both seasons respectively. Root volume characteristic as shown in Table 4, recorded the highest values in the shorter air-layers (150 cm) where combined treatment between IBA (200 ppm) and *B. subtilis* (10 ml) recorded the highest volume with significant increase in comparison with the control (64.12 and 65.28%). Data presented in Table 5 and 6 show that the heaviest fresh and dry roots were detected in the longer air-layers (275 cm) during both seasons (44.00 and 46.33 g fresh weight; 8.19 and 8.63 g dry weight, respectively). Similarly, IBA (200 ppm) and *B. subtilis* (10 ml) combined treatment resulted the heaviest fresh (72.17 and 77.16 g) and dry (12.39 and 13.26 g) roots during the first and second seasons, respectively. A significant interaction between length of air-layers and application substances was observed on root dry weight in both seasons (Table 6). The heaviest dry weights (12.5 and 13.5 g/air-layer) were obtained when the shortest air-layer (150 cm) was treated with IBA (200) plus *B. subtilis* (10 ml).

These results are in agreement with those obtained by Suryanarayana & Rao (1984), Dessalegn & Reddy (2003), Chawla (2011), Reddy et al. (2014) and Gilani et al. (2019). They observed that treating air-layers with IBA accelerate root appearance and improve root characteristics. They added that the response of air-layers to increasing IBA concentrations might be due to the activity of auxin and the accumulation of rooting co-factors at cambial region above the girdled portion. Also, enhancement of auxin concentration in the cell that may be adequate for initiating root primordia early by increasing cell division that further speed up callus formation. The root length determines the ability of the plant to adapt to stress conditions, because longer roots have been associated with high efficiency to assimilate water and nutrients from the soil (Ruiz-Sánchez et al., 2005).



Fig. 1. Main and interaction effects of air-layered branch length, IBA and/or *B. subtilis*.

TABLE 1. Percentage of rooted layers in *C. erectus* as affected by length of air-layering and IBA in combined with *Bacillus subtilis* during the 2017 and 2018 seasons.

| Treatments | Shoot height (cm) | | | | | |
|--|-------------------------------|--------|--------|-------------------------------|--------|--------|
| | 1 st season (2017) | | | 2 nd season (2018) | | |
| | 275 | 150 | Mean | 275 | 150 | Mean |
| Control (water) | 100.00 | 100.00 | 100.00 | 95.00 | 100.00 | 97.50 |
| IBA (200 ppm) | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| <i>B. subtilis</i> (5 ml) | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| <i>B. subtilis</i> (10 ml) | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| IBA (200) + <i>B. subtilis</i> (5 ml) | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| IBA (200) + <i>B. subtilis</i> (10 ml) | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Mean | 100.00 | 100.00 | | 99.20 | 100.00 | |
| LSD at 0.05 | | | | | | |
| Shoot height | | NS | | | NS | |
| Application treatments | | NS | | | NS | |
| Interaction | | NS | | | NS | |

TABLE 2. Root number per rooted layer of *C. erectus* as affected by length of air-layers and the combined treatment of IBA and *B. subtilis* during the 2017 and 2018 seasons.

| Treatments | Shoot length (cm) | | | | | |
|--|-------------------------------|-------|-------|-------------------------------|-------|-------|
| | 1 st season (2017) | | | 2 nd season (2018) | | |
| | 275 | 150 | Mean | 275 | 150 | Mean |
| Control (water) | 13.33 | 33.33 | 23.33 | 14.00 | 34.00 | 23.99 |
| IBA (200 ppm) | 15.00 | 34.67 | 24.84 | 15.83 | 35.02 | 25.42 |
| <i>B. subtilis</i> (5 ml) | 31.00 | 31.67 | 31.36 | 32.55 | 33.89 | 33.21 |
| <i>B. subtilis</i> (10 ml) | 54.00 | 33.33 | 43.67 | 56.65 | 36.66 | 46.66 |
| IBA (200) + <i>B. subtilis</i> (5 ml) | 46.33 | 50.00 | 48.17 | 48.65 | 53.00 | 50.82 |
| IBA (200) + <i>B. subtilis</i> (10 ml) | 56.34 | 53.33 | 54.83 | 59.72 | 57.60 | 58.66 |
| Mean | 35.99 | 39.39 | | 37.89 | 41.69 | |
| LSD at 0.05 | | | | | | |
| Shoot length | | 0.48 | | | 0.47 | |
| Application treatments | | 1.53 | | | 1.63 | |
| Interaction | | 2.17 | | | 2.29 | |

TABLE 3. Root length (cm) per rooted layer of *C. erectus* as affected by length of air-layers and the combined treatment of IBA and *B. subtilis* during the 2017 and 2018 seasons.

| Treatments | Shoot height (cm) | | | | | |
|--|-------------------------------|-------|-------|-------------------------------|-------|-------|
| | 1 st season (2017) | | | 2 nd season (2018) | | |
| | 275 | 150 | Mean | 275 | 150 | Mean |
| Control (water) | 20.33 | 18.67 | 19.50 | 21.35 | 19.04 | 20.20 |
| IBA (200 ppm) | 21.00 | 18.00 | 19.50 | 22.16 | 18.18 | 20.17 |
| <i>B. subtilis</i> (5 ml) | 22.33 | 24.33 | 23.33 | 23.45 | 26.04 | 24.74 |
| <i>B. subtilis</i> (10 ml) | 15.00 | 23.33 | 19.16 | 15.74 | 25.67 | 20.70 |
| IBA (200) + <i>B. subtilis</i> (5 ml) | 15.00 | 25.00 | 20.00 | 15.75 | 26.50 | 21.13 |
| IBA (200) + <i>B. subtilis</i> (10 ml) | 14.67 | 19.67 | 17.17 | 15.55 | 21.24 | 18.40 |
| Mean | 18.06 | 21.50 | | 18.99 | 22.78 | |
| LSD at 0.05 | | | | | | |
| Shoot length | | 1.04 | | | 1.12 | |
| Application treatments | | 1.35 | | | 1.43 | |
| Interaction | | 1.91 | | | 2.02 | |

TABLE 4. Root volume (cm³) per rooted layer of *C. erectus* as affected by length of air-layers and the combined treatment of IBA and *B. subtilis* during the 2017 and 2018 seasons.

| Treatments | Shoot height (cm) | | | | | |
|--|-------------------------------|-------|-------|-------------------------------|-------|-------|
| | 1 st season (2017) | | | 2 nd season (2018) | | |
| | 275 | 150 | Mean | 275 | 150 | Mean |
| Control (water) | 29.00 | 29.00 | 29.00 | 30.45 | 29.58 | 30.02 |
| IBA (200 ppm) | 31.33 | 50.00 | 40.67 | 33.05 | 50.50 | 41.78 |
| <i>B. subtilis</i> (5 ml) | 33.00 | 40.00 | 36.50 | 34.65 | 42.80 | 38.73 |
| <i>B. subtilis</i> (10 ml) | 52.00 | 46.67 | 49.34 | 54.55 | 51.34 | 52.94 |
| IBA (200) + <i>B. subtilis</i> (5 ml) | 62.33 | 68.33 | 65.33 | 65.45 | 72.43 | 68.94 |
| IBA (200) + <i>B. subtilis</i> (10 ml) | 83.33 | 78.33 | 80.83 | 88.33 | 84.60 | 86.46 |
| Mean | 48.50 | 52.05 | | 51.08 | 55.21 | |
| LSD at 0.05 | | | | | | |
| Shoot length | | 1.24 | | | 1.33 | |
| Application treatments | | 1.43 | | | 1.51 | |
| Interaction | | 2.03 | | | 2.13 | |

TABLE 5. Root fresh weight (g/air-layer) of *C. erectus* as affected by length of air-layers and the combined treatment of IBA and *B. subtilis* during the 2017 and 2018 seasons.

| Treatments | Shoot height (cm) | | | | | |
|--|-------------------------------|-------|-------|-------------------------------|-------|-------|
| | 1 st season (2017) | | | 2 nd season (2018) | | |
| | 275 | 150 | Mean | 275 | 150 | Mean |
| Control (water) | 20.67 | 29.00 | 24.84 | 21.70 | 29.58 | 25.64 |
| IBA (200 ppm) | 22.33 | 36.67 | 29.50 | 23.56 | 37.04 | 30.30 |
| <i>B. subtilis</i> (5 ml) | 22.67 | 31.33 | 27.00 | 23.80 | 33.52 | 28.66 |
| <i>B. subtilis</i> (10 ml) | 58.33 | 36.00 | 47.17 | 61.19 | 39.60 | 50.39 |
| IBA (200) + <i>B. subtilis</i> (5 ml) | 62.00 | 58.33 | 60.17 | 65.10 | 61.83 | 63.47 |
| IBA (200) + <i>B. subtilis</i> (10 ml) | 78.00 | 66.33 | 72.17 | 82.68 | 71.64 | 77.16 |
| Mean | 44.00 | 42.94 | | 46.33 | 45.53 | |
| LSD at 0.05 | | | | | | |
| Shoot length | | 0.75 | | | 0.01 | |
| Application treatments | | 1.26 | | | 0.03 | |
| Interaction | | 1.78 | | | 0.04 | |

TABLE 6. Root dry weight (g/air-layer) of *C. erectus* as affected by length of air-layers and the combined treatment of IBA and *B. subtilis* during the 2017 and 2018 seasons.

| Treatments | Shoot height (cm) | | | | | |
|--|-------------------------------|-------|-------|-------------------------------|-------|-------|
| | 1 st season (2017) | | | 2 nd season (2018) | | |
| | 275 | 150 | Mean | 275 | 150 | Mean |
| Control (water) | 4.90 | 4.36 | 4.62 | 5.15 | 4.44 | 4.79 |
| IBA (200 ppm) | 5.00 | 8.03 | 6.52 | 5.28 | 8.11 | 6.69 |
| <i>B. subtilis</i> (5 ml) | 5.64 | 4.44 | 5.04 | 5.92 | 4.75 | 5.34 |
| <i>B. subtilis</i> (10 ml) | 9.92 | 7.33 | 8.63 | 10.41 | 8.06 | 9.24 |
| IBA (200) + <i>B. subtilis</i> (5 ml) | 11.43 | 10.07 | 10.75 | 12.00 | 10.67 | 11.34 |
| IBA (200) + <i>B. subtilis</i> (10 ml) | 12.28 | 12.50 | 12.39 | 13.01 | 13.50 | 13.26 |
| Mean | 8.19 | 7.79 | | 8.63 | 8.26 | |
| LSD at 0.05 | | | | | | |
| Shoot length | | 0.94 | | | 0.97 | |
| Application treatments | | NS | | | NS | |
| Interaction | | 1.09 | | | 1.15 | |

Survival percentage

Data presented in Table 7 show that the best treatments which enhanced survival percentage of successfully rooted air-layers. Generally, treating short or long air-layers with any of IBA (200 ppm) and *B. subtilis* (10 ml), IBA (200 ppm) and *B. subtilis* (5 ml) or *B. subtilis* (10 ml) alone produced the best survival percentages (100%) in both seasons comparing to untreated layers (control). Induction and formation of adventitious roots is a complicated process which depends on several factors including endogenous hormonal level, concentration of carbohydrates and presence of dormant buds (Smart et al., 2003). According to Hartmann et al. (2010), development of adventitious roots passes through a similar process in both cuttings and air layers where roots are developed from the young tissue of the phloem, vascular rays, vascular cambium or callus formed at cutting bases. It should be emphasized that the root characteristics such as volume and length shown by layers is of great importance in the air-layering propagation since they are associated with increasing survivability in field conditions. According to Franco et al. (2005), the improving root volume is necessary to ensure successful installation of the orchard, as it increases the rate of fixation and survival of plants in the field.

Total carbohydrates and phenols content

Data presented in Tables 8 and 9 show that all treatments significantly increased content of total carbohydrates and phenols in layered shoots tissues compared to untreated layers. The highest contents of total carbohydrates (27.15 and 29.32 %) and phenols (48.53 and 52.41 mg Gallic acid equivalent/ g DW) were obtained by treating the shorter air-layers (150 cm) with 200 ppm IBA + *B. subtilis* (10 ml) during both seasons, respectively. Carbohydrates are source of carbon and energy for the biosynthesis of nucleic acids and proteins, and other substances essential to the formation of roots (Lima et al. 2011). One should take into account the fact that the layering need to be integrally connected to the mother plant for all rooting time and that their behavior reflects what happens in the various phases. The increment in polyphenolic compounds produced due to the treatment of IBA plus *B. subtilis* has a direct effect in preventing auxin oxidation and subsequently improving rootability and root characteristics (Mitchell et al., 1986 and Scagel and Linderman, 1998). Also, this effect might be due to that the fact that di- and polyphenol can increase root growth as polyphenol oxidase can induce second -OH group in to the ring of a monophenol (Goodwin, 1976) and convert it into diphenol, which in turn induces better rooting

TABLE 7. Survival percentage of new produced plants after 3 months from transplanting of *C. erectus* affected by length of air-layers and the combined treatment of IBA and *B. subtilis* during the 2017 and 2018 seasons.

| Treatments | Shoot height (cm) | | | | | |
|--|-------------------------------|-------|-------|-------------------------------|-------|-------|
| | 1 st season (2017) | | | 2 nd season (2018) | | |
| | 275 | 150 | Mean | 275 | 150 | Mean |
| Control (water) | 65 | 80 | 72.50 | 72 | 90 | 81 |
| IBA (200 ppm) | 90 | 92 | 91 | 100 | 95 | 97.50 |
| <i>B. subtilis</i> (5 ml) | 95 | 98 | 96.50 | 92 | 97 | 94 |
| <i>B. subtilis</i> (10 ml) | 100 | 100 | 100 | 100 | 100 | 100 |
| IBA (200) + <i>B. subtilis</i> (5 ml) | 100 | 100 | 100 | 100 | 100 | 100 |
| IBA (200) + <i>B. subtilis</i> (10 ml) | 100 | 100 | 100 | 100 | 100 | 100 |
| Mean | 91.67 | 95.00 | | 94.00 | 97.00 | |
| LSD at 0.05 | | | | | | |
| Shoot length | | 1.24 | | | 0.83 | |
| Application treatments | | 2.41 | | | 1.03 | |
| Interaction | | 3.41 | | | 1.46 | |

(Kunal and Syamal, 2005). Phenolic compounds are shown to interact with proteins leading to altered metabolism which form roots in air-layers (Kefeli and Kutacek, 1976).

The current experiment proved that root formation of buttonwood air-layers could be improved by the application of IBA and/or *B. subtilis*. The treatment of IBA (200 ppm) + bacillus (10 ml) resulted in the highest values of rooting characteristics; root number, root length, root volume, root fresh weight and root dry weight in

addition to survival %, carbohydrates % and total phenolics. The positive correlation among most of these characteristics as indicated in Table 10 could explain the enhancement in overall root formation obtained by the IBA (200 ppm) and Bacillus (10 ml) treatment. For example root number, as an important characteristic of *C. erectus* air-layers, is positively and significantly correlated with all rooting and survival characteristics in addition to total carbohydrates and phenolics.

TABLE 8. Total carbohydrates (%) of *C. erectus* as affected by length of air-layers and the combined treatment of IBA and *B. subtilis* during the 2017 and 2018 seasons.

| Treatments | Shoot height (cm) | | | | | |
|--|-------------------------------|-------|-------|-------------------------------|-------|-------|
| | 1 st season (2017) | | | 2 nd season (2018) | | |
| | 275 | 150 | Mean | 275 | 150 | Mean |
| Control (water) | 24.28 | 24.16 | 24.22 | 25.49 | 24.64 | 25.07 |
| IBA (200 ppm) | 25.15 | 25.44 | 25.29 | 26.53 | 25.69 | 26.11 |
| <i>B. subtilis</i> (5 ml) | 25.19 | 25.18 | 25.19 | 26.45 | 26.94 | 26.70 |
| <i>B. subtilis</i> (10 ml) | 25.36 | 25.67 | 25.52 | 26.61 | 28.24 | 27.42 |
| IBA (200) + <i>B. subtilis</i> (5 ml) | 25.48 | 26.82 | 26.15 | 26.75 | 28.43 | 27.59 |
| IBA (200) + <i>B. subtilis</i> (10 ml) | 25.93 | 27.15 | 26.54 | 27.49 | 29.32 | 28.40 |
| Mean | 25.23 | 25.74 | | 26.55 | 27.21 | |
| LSD at 0.05 | | | | | | |
| Shoot length | | 0.28 | | | 0.30 | |
| Application treatments | | 0.64 | | | 0.66 | |
| Interaction | | NS | | | 0.94 | |

TABLE 9. Total phenolics (mg GAE/ g DW) of *C. erectus* as affected by length of air-layers and the combined treatment of IBA and *B. subtilis* during the 2017 and 2018 seasons.

| Treatments | Shoot height (cm) | | | | | |
|--|-------------------------------|-------|-------|-------------------------------|-------|-------|
| | 1 st season (2017) | | | 2 nd season (2018) | | |
| | 275 | 150 | Mean | 275 | 150 | Mean |
| Control (water) | 22.73 | 24.78 | 23.75 | 23.86 | 25.27 | 24.57 |
| IBA (200 ppm) | 25.87 | 28.84 | 27.36 | 27.29 | 29.13 | 28.21 |
| <i>B. subtilis</i> (5 ml) | 31.03 | 35.31 | 33.17 | 32.58 | 37.78 | 35.18 |
| <i>B. subtilis</i> (10 ml) | 37.10 | 44.04 | 40.57 | 38.92 | 48.44 | 43.68 |
| IBA (200) + <i>B. subtilis</i> (5 ml) | 32.78 | 36.90 | 34.84 | 34.42 | 39.11 | 36.77 |
| IBA (200) + <i>B. subtilis</i> (10 ml) | 38.14 | 48.53 | 43.36 | 40.43 | 52.41 | 46.42 |
| Mean | 31.28 | 36.40 | | 32.92 | 38.69 | |
| LSD at 0.05 | | | | | | |
| Shoot length | | 2.57 | | | 2.71 | |
| Application treatments | | 1.74 | | | 1.84 | |
| Interaction | | 2.47 | | | 2.61 | |

TABLE 10. Correlation coefficients matrix (r) of rooting characteristics of *C. erectus* as affected by length of air-layers and the combined treatment of IBA and *B. subtilis* during the 2017 and 2018 seasons.

| Characteristics | Season | Root % | Root number/ plant | Root length/ plant | Root volume/ plant | Root fw/plant | Root dw/plant | Survival % | Total carbohydrate |
|--------------------|-----------------|--------|-----------------------|-----------------------|-----------------------|---------------|---------------|------------|-----------------------|
| Root number/plant | 1 st | 0.53** | - | - | - | - | - | - | - |
| | 2 nd | 0.52** | - | - | - | - | - | - | - |
| Root length/plant | 1 st | -0.05 | -0.41* | - | - | - | - | - | - |
| | 2 nd | -0.02 | -0.34* | - | - | - | - | - | - |
| Root volume/plant | 1 st | 0.35* | 0.86** | -0.33 | - | - | - | - | - |
| | 2 nd | 0.34* | 0.87** | -0.25 | - | - | - | - | - |
| Root fw/plant | 1 st | 0.36* | 0.93** | -0.49** | 0.95** | - | - | - | - |
| | 2 nd | 0.36* | 0.93** | -0.42* | 0.95** | - | - | - | - |
| Root dw/plant | 1 st | 0.30 | 0.85** | -0.47** | 0.93** | 0.94** | - | - | - |
| | 2 nd | 0.29 | 0.85** | -0.39* | 0.94** | 0.94** | - | - | - |
| Survival % | 1 st | 0.82** | 0.71** | -0.02 | 0.63** | 0.62** | 0.59** | - | - |
| | 2 nd | 0.88** | 0.63** | -0.04 | 0.57** | 0.57** | 0.53** | - | - |
| Total carbohydrate | 1 st | 0.39** | 0.62** | 0.11 | 0.75** | 0.63** | 0.69** | 0.67** | - |
| | 2 nd | 0.31 | 0.56** | 0.32 | 0.71** | 0.59** | 0.63** | 0.57** | - |
| Total phenolics | 1 st | 0.44** | 0.69** | 0.05 | 0.71** | 0.64** | 0.64** | 0.74** | 0.74** |
| | 2 nd | 0.41* | 0.69** | 0.17 | 0.72** | 0.64** | 0.65** | 0.61** | 0.86** |

* Significant at $p \leq 0.05$, ** Significant at $p \leq 0.01$

Conclusion

The layering in buttonwood, when prepared from the 150 cm length branches provide the best results of rooting, verified by the high percentage of rooted layers, as well as by higher mass, length, number and volume of roots compared with 275 cm length ones. IBA combined with *B. subtilis* (10 ml) had the best results of rooting during both seasons, compared to the other treatments and control. However, when the shorter layers of buttonwood was treated with IBA in combined with *B. subtilis* (10 ml), high quality of rooting was obtained.

Acknowledgment

I express my thanks to Dr. Omer Hosni Mohamed Ibrahim for his assistance in the chemical analysis in this study.

Funding statement

I express my appreciation to the Ornamental Plants and Landscape Gardening Department, Faculty of Agriculture, Assiut University for supplying the chemicals and apparatus for this research work.

Conflict of interest

The author declare that they have no conflict of interest.

References

- Arshad, M. and Frankenberger, W.T. (1998) Plant growth regulating substances in the rhizosphere: microbial production and functions. *Adv. Agron.*, **62**, 146–151.
- Audus, L.J. (1972) Plant Growth Substances. In: *Chemistry and Physiology. A Plant Science Monograph*, vol. I, Publ. Polunin, N. (Ed.), Leonard Hill Book, London, pp. 146-154.
- Bose, T.K., S. Mitra, K., Sadhu, M.K. and Das, P. (1997) "Propagation of tropical and sub-tropical horticultural crops", Revised edn., Nayaprakash, Calcutta, India, pp. 662.
- Branney, P. (1989) "Propagation of tree species for afforestation in northern Sudan", Northern Region Irrigation Project (NRIRP), Forestry Development Component. Overseas Development Administration, London, UK, p. 42.

- Campbell, N.A., Reece, J.B. and Mitchell, L.G. (1999) "Biology", 5th ed., Manlow Park, CA, Addison, Wesley, Longman, Inc., 1175 p.
- Chawla, W. (2011) Studies on air layering, survival and growth of rooted layers in litchi (*Litchi chinensis* Sonn.). *M.Sc. Thesis*, Fac. Agric., Univ. Hort. and Fort., Nauni, Solan, India. <http://krishikosh.egranth.ac.in/handle/1/5810001296>.
- Dessalegn, Y. and Reddy, Y.N. (2003) Effects of different concentrations of auxins on rooting and root characters of air and ground layers of jojoba (*Simmondsia chinensis* (LINK.) C. K. Schneider). *Ethiop. J. Sci.*, **26**, 155-159.
- Fales, F.W. (1951) The assimilation and degradation of carbohydrates by yeast cells. *J. Bio. Chem.*, **193**, 113-124.
- Franco, C.F., Prado, R.M., Braghirolli, L.F., Leal, R.M., Perez, E. and Romualdo, L.M. (2005) Use of pruning and different air laying diameters on plant development and nutrient accumulation in litchi. *Revista Brasileira de Fruticultura. Jaboticabal*, **27**, 491-494.
- Gilani, S.A.Q., Shah K., Ahmed I., Basit A., Sajid M., Bano A.S., Ara G. and Shahid U. (2019) Influence of indole butyric acid (IBA) concentrations on air layer age in guava (*Psidium guajava* L.) cv. Sufeda. *Pure Appl. Biol.*, **8**, 355-362.
- Gliman, E.F and Watson, D.G. (1993) *Conocarpus erectus*: Buttonwood. Fact sheet ST-179. U.S. Forest Service and Southern Group of State Foresters. *Gainseville, FL.*, 3p. (Available online: <http://hort.ufl.edu/trees/conocarpus.pdf>, Accessed on 1/5/2019).
- Gomez, K.A. and Gomez, A.A. (1984) "Statistical Procedures for Agricultural Research", 2nd ed., John Wiley, NY, 680 p.
- Goodwin, T.W. (1976) *Chemistry and Bio-chemistry of Plant Pigments*. Academic Press, 1, 736.
- Harrington, J.T., Mexal, J.G., Fisher, J.T. (1994) Volume displacement provides a quick and accurate way to quantify new root production. *Tree Plant Notes*. **45**, 121-124.
- Hartmann, H.T., Kester, D.E., Davies Júnior, F.T. and Geneve, R.L. (2010) "Plant Propagation: Principles and Practices", 8th ed., New Jersey, Prentice Hall, 915 p.
- Kefeli, V.I. and Kutacek, M. (1976) Phenolic substances and their possible role in plant growth regulation. In: Pilet, P.E. (ed.) *Plant Growth Regulation, Proceedings in Life Sciences*, Springer, Berlin, Heidelberg, pp. 181-182.
- Khalid, A., Tahir, S., Arshad, M. and Zahir, Z.A. (2004) Relative efficiency of rhizobacteria for auxin biosynthesis in rhizosphere and non-rhizosphere soils. *Aus. J. Soil Res.*, **42**, 921-926.
- Khamna, S., Yokota, A., Peberdy, J.F. and Lumyong, S. (2010) Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. *EurAsia J. Bio. Sci.*, **4**, 23-32.
- Kunal, K. and Syamal M.M. (2005) Effect of etiolation and plant growth substances on rooting and survival of air layering of guava. *Ind. J. Hort.*, **62**, 290-292.
- Leveau, J.H.J. and Lindow, S.E. (2005) Utilization of the plant hormone indole-3-acetic acid for growth by *Pseudomonas putida* strain 1290. *Appl. Environ. Microbiol.*, **71**, 2365-2371.
- Lima, D.M., Biasi, L.A., Zanette, F., Zuffellato-Ribas, K.C., Bona, C., Mayer, J.L.S. (2011) Rooting capacity of *Maytenus muelleri* Schwacke cuttings with indolebutyric acid application related to anatomical aspects. *Revista Brasileira de Plantas Medicinai, Botucatu*, **13**, 422-438.
- Maliauskas, G., Venskutonis, P.R. and Van Beek, T. A. (2004) Screening of Radical Scavenging Activity of Some Medicinal and Aromatic Plants Extracts. *Food Chem.*, **85**, 231-237.
- Mergen, F. (1953). Air layering as a possible method to reproduce selected Slash Pine. *Naval Stores Review*, **63**, 19-20.
- Mitchell, R.J., Garrett H.E., Cox, G.S. and Atalay, A. (1986) Boron and ectomycorrhizal influences on indole-3-acetic acid levels and indole-3-acetic acid oxidase and peroxidase activities of *Pinus echinata* roots. *Tree Physiol.*, **1**, 1-8.

- Popp, M., Lüttge, U., Cram, W.J., Diaz, M., Griffiths, H., Lee, H.J.S., Medina, E., Schäfer, C., Stimmel, K.H. and Thonke, B. (1989) Water relations and gas exchange of mangroves. *New Phytologist.*, **111**, 293-307.
- Reddy, P.P.N., Ray, N.R., Patel, A.D. and Patel, J.S. (2014) Effect of rooting media and IBA (indole butyric acid) levels on rooting and survival of air layering in fig (*Ficus carica* L.) cv. POONA under middle Gujarat agro-climatic conditions. *The asian J. Hort.*, **9**, 1-5.
- Ruiz-Sánchez, M.C., Plana, V., Ortuño, M.F., Tapia, L.M. and Abrisqueta, J.M. (2005) Spatial root distribution of apricot trees in different soil till age practices. *Plant and Soil, Dordrecht*, **272**, 211-221.
- Scagel, C.F. and Linderman, R.G. (1998) Influence of ectomycorrhizal fungi inoculation on growth and root IAA concentrations of transplanted conifers. *Tree Physiol.*, **18**, 739-747.
- Smart, D.R., Kocsis, L., Walker, M.A. and Stockert, C. (2003) Dormant buds and adventitious root formation by *Vitis* and other woody plants. *J. Plant Growth Regulation, New York*, **21**, 296-314.
- Suryanarayana, V. and Rao K.V. (1984) Effect of growth regulators on air layering of fig and pomegranate. *Andra Agric. J.*, **31**, 54-58.
- Waisel, Y., Ashel, A. and Kafkafi, U. (1991) "Plant roots: the hidden half", New York, March dekker, Inc., 848 p.

إنتاج ترافيد هوائية عالية الجودة من نبات الكونوكاريس إستجابة لاندول حامض البيوتريك وبكتريا باسيليس ساتليس

عصام يوسف عبدالحفيظ

قسم الزينة وتنسيق الحدائق - كلية الزراعة - جامعة أسيوط - أسيوط - مصر.

أجريت الدراسة الحالية بهدف تقييم تأثير الاختلاف في أطوال الأفرع المستخدمة في الترافيد الهوائية والمعامله باندول حامض البيوتريك منفرداً أو مشتركاً مع التلقيح بمعلق بكتريا الباسيليس ساتلس على السلوك التجذيري لنباتات الكينوكاريس. اشتملت التجربة على طولين من الترافيد الهوائية (٢٧٥، ١٥٠ سم) وست معاملات من اندول حامض البيوتريك (٢٠٠ جزء في المليون) وبكتريا الباسيليس ساتلس (٥ أو ١٠ مل/ترقيده وكان عدد الخلايا في المعلق البكتيري ١٠^٨) المضافة إلى بيئة التجذير حيث كانت منفردة أو في توليفات مختلفة. قيمت استجابة ترافيد الكينوكاريس بالنسبة للمعاملات على أساس الصفات الجذرية التي أظهرت أفضل النتائج عند المعاملة باندول حامض البيوتريك مشتركاً مع بكتريا الباسيليس ساتلس كالتالي: أعلى نسبة تجذير (١٠٠٪)، أكبر عدد جذور لكل ترقيده (٥٨،٥٦)، أطول جذر لكل ترقيده (٢٦،٥٠ سم)، أعلى حجم جذري لكل ترقيده (٨٦،٤٦ سم^٣)، أكبر وزن طازج للجذور (٨٢،٢٨ جم)، أكبر وزن جاف للجذور (١٣،٥٠ جم) و أعلى نسبة ترافيد ناجحة كونت جذور (١٠٠٪) بالإضافة إلى أعلى محتوى كلى للكربوهيدرات (٢٨،٤٠٪) وكذلك أعلى محتوى للفينولات الكلية (٥٢،٤١ مجم مكافئ حامض الجاليك/جم وزن جاف). نستخلص من النتائج أن الترافيد الهوائية الأقصر (١٥٠ سم) المعاملة ب ٢٠٠ جزء في المليون من اندول حامض البيوتريك مضافاً إليه بكتريا الباسيليس (١٠ مل/ترقيده) ساتلس أظهرت تفوقاً معنوياً على جميع المعاملات الأخرى.