

Propagation of Hardwood Cuttings of Some *Ficus* species as Affected by Microorganisms and Compost Tea Treatments

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Abstract: *Ficus* genus one of the most important trees propagated principally by stem cuttings. In recent years Plant growth promoting rhizobacteria (PGPR) and organic matter are used as alternative systems to produce rooted cuttings. This study was conducted to evaluate the effect of mixture of PGPR (*Bacillus polymyxa*, *Rhizobium leguminosarum* and *Pseudomonas fluorescens*), compost tea and 4000ppm IBA as control on rooting ability of eight species of *Ficus* woody genus in the two seasons. Mixture of PGPR were more effective than most of the other treatments in increasing rooting percentage, roots length and number of root, whereas compost tea was the best treatment in number of shoot and leaves. On the other hand, *F. pyriformis* recorded highest rooting percentage, root length and number of shoot than other species. *F. hispida* and *F. filutea* recorded the best response in number of root, whereas *F. hispida* produced highest number of leaves. On commercial scale use of PGPR and compost tea for rooting of *Ficus* genus can reduce the use of synthetic auxins like IBA.

Key words: *Ficus* · Stem cutting · Plant growth promoting rhizobacteria (PGPR) · IBA · Compost tea

INTRODUCTION

The genus *Ficus* has about 1000 species of trees, shrubs or vines, commonly called figs, belong to the family Moraceae. It is distributed in tropical and subtropical regions. *Ficus* species, as *F. nitida*, *F. benjamina* and *F. hawaii*, have multiple uses as indoor ornamental plants and garden or roadside trees [1,2]. *Ficus* is an important ornamental plant for the nurserymen. It is generally propagated by air layering and tip cuttings, the plants propagated through air layering are small in quantity, need more skill and time. So the easiest and economic method is mass production through cutting. On the other hand, the success of rooting of woody stem cuttings depends mainly on the physiological stage of the mother plant, the time of planting of the cuttings and the type of growth regulators used. Therefore, a key step in vegetative propagations is adventitious root formation losses occur because of the poor quality of the root system or of the shoot and because of poor or slow rooting. Auxins are commonly used to stimulate root initiation in plant [3]. Also, Memon *et al.* [4] stated

that NAA increased rooting percentage and root number of *Ficus elastic* and *Bougainvillea*. Moreover, Karimiyan *et al.* [5] reported that the highest number of roots per rotted cutting was obtained by 6000 ppm IBA treatment with being significantly different from all other treatments and the control on the rooting ability of *Ficus benjamina* and *Syringaa murensis*.

Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria which have the ability to colonize the roots and either promotes plant growth through direct action or via biological control of plant diseases [6]. The mechanisms involved in plant growth promotion by PGPR involve direct and indirect effects. Direct effects occur when PGPR produces substance such as phytohormones. These microorganisms are the potential tools for sustainable agriculture and the trend for future [7]. One of the more characteristic effects of PGPR is an increased root elongation rate and perhaps initiation rate of lateral roots resulting in more branched root system Compost tea is a liquid extract of compost made by steeping the compost in water for various time periods. Compost tea is suppress disease by promoting the proliferation of

beneficial microbes, which then act as a biological control over pathogens [8]. One hypothesis is that the physical and chemical properties of the nutrients in compost tea, the humic components in the compost tea, or a combination of the nutrients and humic components may improve the nutritional status of plants, be directly toxic to the pathogen and/or induce systemic resistance to the pathogen [9]. The development of vegetative propagation methods is a useful tool for the conservation of genetic resources and for the implementation of domestication programs.

The aim of this study is to investigate the effect of PGPR and compost tea as compared with Indole butyric acid (IBA) to improve a commercial propagation protocol for eight different *Ficus* species as well as minimizing the use of synthetic auxins.

MATERIALS AND METHODS

Plant Materials and Rooting Media: Experiments were carried out at the National Gene Bank greenhouse (ARC) Giza, Egypt. The source of mother plants of *Ficus* species grown in the Orman Botanical Garden (Ministry of Agriculture, Giza, Egypt) since 1875 until now. Hard wood stem cuttings of *Ficus* species (Table, 1) were prepared from middle parts of vigorous shoots of above 50 year old plants during February-March in both 2014 and 2015 experimental seasons. The cuttings had (3-4 buds/cutting), 20 cm in length and 1 cm in diameter. The first treatment, cuttings were dipped in 10 ml mixture of PGPR at the concentration of 10^9 cfu ml⁻¹ for 30 min. The second treatment, The cuttings were dipping in extract of compost tea for 30 min. Cuttings in the control group were treated with IBA at 4000 ppm for 5 min., The cuttings were cultivated in black polyethylene bags (20 cm x 20 cm) filled with mixture of sand and peatmoss (1:1 v/v) medium. Each bag contained three cuttings at a depth of 5 cm under plastic tunnel in a greenhouse maintained at 21± 2 °C.

Table 1: List of *Ficus* species plant materials used in present investigation.

Accession number	Scientific name	Family	Native
12194	<i>Ficus afzelii</i>	Moraceae	Tropic Africa
12195	<i>Ficus asperrima</i>	Moraceae	Indian
12196	<i>Ficus hispida</i>	Moraceae	Tropic Asia
12197	<i>Ficus lutea</i>	Moraceae	Tropic Asia
12198	<i>Ficus platiboda</i>	Moraceae	Tropic Asia
12199	<i>Ficus pyriformis</i>	Moraceae	Tropic Asia
12200	<i>Ficus spragueana</i>	Moraceae	Tropic Asia
12201	<i>Ficus trijuja</i>	Moraceae	Tropic Asia

Data were collected after 3 months and the evaluated parameters were rooting percentage, the number of main roots per cutting, average root length, number of leaves per cutting and the number of shoot/cutting.

Bacterial Strains and Their Plant Growth Regulators

Roles: Bacterial strains of plant growth promoting rhizobacteria were obtained from Biofertilizers Production Unit, Agric. Microbio., Res. Dept., Soils, Water and Environ. Res. Inst.,(SWERI), Agric. Res. Center (ARC) Giza, Egypt, (*Bacillus polymyxa*, *Rhizobium leguminosarum* and *Pseudomonas fluorescens*).

Estimation of IAA according to Gordon and Weber [10] the synthesis of IAA was estimated using FeCl₃-HClO₄. Bacterial strains were grown on LB media supplemented with 5mM L- Tryptophan, incubated for 24 h, thereafter IAA production was estimated.

Siderophores Production: Siderophores production was detected by growth on CAS medium [11] and O.D was measured at 630 nm [12].

Phosphate Solubilization: The ability to solubilize calcium phosphate was tested according to Mehta and Nautiyal [13].

Compost Tea: Compost tea is a liquid extract of compost made by steeping compost in water (1:10) for up to 14 days to extract nutrients that when applied to plant promotes health and vitality [14]. The chemical composition of compost tea showed in (Table, 2). Compost was obtained from Biofertilizers Production Unit, Agric. Microbio., Res. Dept., SWERI, ARC, Giza, Egypt.

Data Analysis: The experimental design used was a one way ANOVA with three replicates of each treatment. All data were subjected to statistical analysis according to the procedures reported by Snedecor and Cochran [15] and means were compared by Duncan's multiple range tests at 5% level of probability in the two seasons after arcsine transformation of rooting percentage.

Table 2: The chemical composition of compost tea

Chemical composition	Value
pH	6.91
Organic carbon (%)	5.80
Organic matter (%)	9.98
Total nitrogen (%)	0.053
Total soluble nitrogen (ppm)	131.7
Available -P (ppm)	41.3
Available - K (ppm)	380.9
Humic acid g kg ⁻¹ dw	10.8

RESULTS AND DISCUSSIONS

The results of selected bacterial strains were screened in (Table, 3) showed that most of the tested PGPR strains were able to produce IAA, siderophores and solubilizing phosphorus in great variation. The amount of IAA produced varied among bacteria, ranging from 59 (*Rhizobium leguminosarum*) to 108.1 $\mu\text{g ml}^{-1}$ (*Pseudomonas fluorescens*). Many microorganisms that interact with plants can produce plant growth regulators such as auxins. Among them, auxin is one of the most well known phytohormones because of its role in the initial process of lateral and adventitious root formation. The endogenous level of IAA in the plant is also important for successful rooting [16]. *Rhizobium leguminosarum* recorded the highest strains in siderophores production followed by *Bacillus polymyxa* and *Pseudomonas fluorescens* (60.13^a, 31.02^b, 14.82^c), respectively. Whereas *Bacillus polymyxa* exhibited the best results for phosphate solubilization.

Most reports concentrated on the effect of certain factors on plant production *via* cuttings for one species or other species. Premixed formulations containing plant growth factors including the hormones, indole-3-butyric acid and paclobutrazol, the nutrients N,P, K and trace elements and the protecting agent sportac, improved rooting from cutting and promoted the development of the root system [17].

Rooting Percentage: The effect of PGPR; Compost tea and IBA treatments and different *Ficus* species on the rooting percentage of *Ficus* hardwood cuttings prepared from 15th February till 15th March are presented in (Table, 4). Results indicated that, treating *Ficus* species hardwood cuttings with PGPR gave the highest significant rooting percentage (39.38 %) compared with other treatments. On the other hand, *Ficus* species exhibit that the *F. pyriformis* resulted greater value of rooting percentage (78.34 %) followed by *F. asperima* (36.39 %) and *F. hispida* (34.45 %), respectively compared with other species. While, others *F. afzelii*, *F. lutea*, *F. platiboda* and *F. spragueana* rooted consistently poorly.

Table 3: Plant growth promoting properties of strains.

Bacterial strain	IAA ($\mu\text{g ml}^{-1}$)	Siderophore (%)	Phosphate solubilizing
<i>Rhizobium leguminosarum</i> (bv.viciae)	59 C	60.13 a	-
<i>Pseudomonas fluorescens</i>	108.1 A	14.82 c	+
<i>Bacillus polymyxa</i>	71 B	31.02 b	++

Table 4: Specific effects of Indole butyric acid, PGPR and compost tea on rooting (%), average root length (cm), number of root per cutting, number of leaves per cutting and number of shoot of *Ficus* species stem cuttings (Average of 2014 and 2015 seasons).

Treatments	Rooting (%)	Root Length(cm)	Number of root Per cutting	No. of leaves Per cutting	Number of shoot
Growth regulator					
IBA 4000 ppm	19.38c	5.67 c	10.90c	16.65b	2.12b
PGPR	39.38a	14.50a	13.53a	16.23b	2.54ab
Compost tea	32.29b	10.99b	11.67b	24.25a	3.62a
LSD 0.05	5.010	3.444	4.582	5.533	1.309
Species					
<i>Ficus afzelii</i>	16.67c	8.47c	6.61c	5.16d	0.94e
<i>Ficus asperima</i>	36.39b	8.16c	10.58bc	20.28b	3.55bc
<i>Ficus hispida</i>	34.45b	13.34b	18.08a	31.00a	3.89ab
<i>Ficus lutea</i>	12.78c	9.88bc	18.22a	13.61c	1.66de
<i>Ficus platiboda</i>	16.11c	3.48d	8.33c	19.34b	2.39cd
<i>Ficus pyriformis</i>	78.34a	20.77a	14.61ab	21.89b	5.00a
<i>Ficus spragueana</i>	18.61c	9.05c	10.5bc	19.84b	1.67de
<i>Ficus trijuja</i>	29.45b	9.93bc	9.27c	21.22b	3.00bcd
LSD 0.05	5.010	3.444	4.582	5.533	1.309

Mean followed by the similar letter(s) are not significantly different by Duncan test ($P < 0.05$).

Table 5: Interaction effects of Indole butyric acid, PGPR and compost tea and their combinations on rooting (%), average root length (cm), number of root per cutting, number of leaves per cutting and number of shoot of *Ficus* species stem cuttings (Average of 2014 and 2015 seasons).

Treatments	G.R	Rooting (%)	Root Length (cm)	Number of root	No. of leaves	Number of shoot
				Per cutting	Per cutting	
Species						
<i>F. afzelii</i>	IBA	0.00o	0.00J	0.00L	0.00I	0.00h
	PGPR	36.72f	15.00cd	8.00ijk	10.17i-l	1.66e-g
	Compost tea	22.04klm	10.43ef	11.83e-i	5.33kl	1.16gh
<i>F. asperrima</i>	IBA	19.79lmn	2.25hij	11.42f-j	9.50jk	2.33d-g
	PGPR	49.81d	11.92de	4.83k	13.67g-j	1.83efg
	Compost tea	38.68ef	10.33ef	15.50c-f	37.67b	6.50b
<i>F. hispida</i>	IBA	31.05ghi	4.66ghi	18.17bcd	21.83d-f	3.66cd
	PGPR	33.70fgh	21.5a	15.25c-f	24.33c-e	4.33c
	Compost tea	42.62e	13.85cde	20.83ab	46.83a	3.66cd
<i>F. lutea</i>	IBA	17.20mn	4.16ghi	16.83b-e	19.50e-g	1.83efg
	PGPR	21.29lm	12.5de	25.17a	9.16jk	1.16gh
	Compost tea	23.18kl	13.0cde	12.67e-i	12.17h-j	2.00efg
<i>F. platiboda</i>	IBA	14.90n	1.63ij	6.16jk	16.83f-h	1.50fg
	PGPR	25.28jkl	6.66g	9.83g-k	14.83g-j	2.50d-g
	Compost tea	28.96hij	2.15hij	9.00h-k	26.33cd	3.16cde
<i>F. pyriformis</i>	IBA	55.32c	21.33a	15.30c-f	13.17g-j	3.00c-f
	PGPR	61.76b	19.40ab	14.00d-h	16.17f-i	3.83cd
	Compost tea	71.66a	21.58a	14.50d-g	36.33b	8.16a
<i>F. spragueana</i>	IBA	27.12ijk	5.50ghi	11.83e-i	35.17b	2.33d-g
	PGPR	36.22fg	21.67a	19.83bc	24.33cde	2.66d-g
	Compost tea	0.00o	0.00J	0.00I	0.00I	0.00h
<i>F. trijuja</i>	IBA	20.64lm	5.83gh	7.50ijk	17.17f-h	2.33d-g
	PGPR	42.59e	7.37fg	11.33f-j	17.17f-h	2.33d-g
	Compost tea	33.13fgh	16.58bc	9.00h-k	29.33c	4.33c
LSD 0.05		5.010	3.444	4.582	5.533	1.309

Mean followed by the similar letter(s) are not significantly different by Duncan test ($P < 0.05$).

Interaction between *Ficus* species and treatments including (plant growth promoting (PGPR), compost tea and IBA) showed that the rooting (%) of PGPR, compost tea and IBA treatments varied between 0.0 and 71.66 % (Table, 5). *F. pyriformis* had the best significantly species in rooting (%) under different three treatments, compost tea had (71.66 %), PGPR (61.76 %) and IBA as control had (55.32 %), respectively. Meanwhile, no rooting was observed on the control cutting with *F. afzelii* (0.0 %).

Root Length (cm): The root length of eight species under three treatments is summarized in (Table, 4) the PGPR recorded the best treatment (14.50 cm) followed by compost tea (10.99 cm). However, *F. pyriformis* recorded the highest results (20.77 cm) and *F. hispida* (13.34 cm). Meanwhile, one genotype (*F. platiboda*) had the lowest

root length (3.48 cm). the rest species showed intermediate root length. The interaction effect between *Ficus* species and treatments showed that three species had the highest significant value *F. spragueana* (21.67 cm), *F. pyriformis* (21.58 cm) and *F. hispida* (21.50 cm), respectively under PGPR and compost tea treatments compared with other species and treatments (Table, 5).

Number of Root per Cutting: In respect of the number of root, data in (Table, 4) revealed that, significant differences between all the used treatments cuttings treated with PGPR produced the highest number of roots (13.53) per rooted cutting, while, control treated cuttings produced the lowest number of roots (10.90) per rooted cutting. Meanwhile, the species recorded significant value, *F. hispida* (18.08) and *F. lutea* (18.22) had the

highest number of root/cutting followed by *F. pyriformis* (14.61). While, *F. afzelii* had lowest number of root/cutting (6.61). On the other hand, the interaction between three treatments and eight species illustrated high significant increase in number of root, *F. lutea* recorded the highest value (25.17) under the PGPR treatment followed by *F. hispida* (20.83) under compost tea treatment (Table, 5).

Number of Leaves per Cutting and Number of Shoot:

Concerning the effect of PGPR, Compost tea and IBA, such three treatments produced significantly higher values in both seasons for each of transplants number of leaves and number of shoot. As shown in (Table, 4) among these three treatments, the use of compost tea and PGPR gave significantly better results for the number of leaves and number of shoot. However, IBA treatment ranked the second, this concern. Numerically, the increase in transplant leaves number and shoot number due to compost tea reached (24.25), (3.62), respectively. On the other hand, *F. hispida* recorded the best number of leaves (31.00). Meanwhile, *F. pyriformis* have the highest number of shoot (5.00). The interaction between *Ficus* species and plant growth promoting exhibit *F. hispida* under compost tea recorded the highest number of leaves (46.83) followed by *F. asperrima* (37.67), *F. pyriformis* (36.33) under the same treatment while, *F. afzelii* and *F. spragueana* had the lowest number of leaves under the IBA treatment (Table, 5). However number of shoot data shown in (Table, 5) *F. pyriformis* had the highest number of shoot (8.16) under compost tea treatment followed by *F. asperrima* had (6.5) under the same treatment. Meanwhile, two species (*F. afzelii* and *F. spragueana*) had the lowest number of shoot under IBA and Compost tea.

The rooting parameters on cuttings of *Ficus* under study were activated corresponding with the effect of tested treatments. The PGPR treatment have the ability to produce IAA, siderophores and soluble phosphorous. Plant hormones regulate a range of cellular and physiological process, as cell division, cell enlargement, bud dormancy and flowering auxin, one of the plant hormones, stimulates differentiation of phloem and xylem, root initiation on stem cutting and development of branch roots Lwin *et al.* [18] and Kasem and Abd El-Baset [19] studied the effect of *Agrobacterium rhizogenes* on *Lavandula angustifolia* and reported, the treatment have ability to increase the effect on nitrogen content, carbohydrates and phenols which succeeded to form adventitious roots of higher qualities. It is evident that the

treatment of PGPR on cuttings of different plant species showed genotype dependent rooting and increased root growth [20, 21]. Patten and Glick [22] reported that IAA producing *Pseudomonas putida* increased the length of canola seedling roots on average 35 to 50 %. Therefore, these results could be important for particularly difficult to root woody plants, in our case *Ficus spp.*, in order to obtain higher rooting percentages by inoculation with bacteria. McAfee *et al.* [23] showed that rooting of *Pinus* was higher when they were inoculated with bacteria strains. Esitken *et al.* [24] tested PGPR for rooting in rose hip and sour cherry cuttings and found that PGPR were effective to obtain high rooting percentages. Caesar and Burn [25] observed that seedling of apple gave better lateral roots when treated with PGPR. Pane *et al.* [26] recoded that compost tea rich in soluble mineral nutrients and contains a high population of microbiota can be used to fertilize crops; inoculate crop residue to facilitate decomposition; improve nutrient cycling in soil through increased microorganism activity and manage certain plant pathogens, to some extent, through microbial competition; improved plant nutrition and build soil structure. Humic acids (HA), can be used in cuttings of ornamental plants also promoting rooting. Humic acids can promote plant growth and development in several plants of agronomic interest. These effects reflect in the increase of root growth rate, increases in plant biomass and root architecture changes. Stimulating effects on shoot area should also be highlighted since it increases the accumulation of foliar nutrients and chlorophyll biosynthesis [9].

CONCLUSION

This study demonstrated that the mixture of PGPR belonging to genus (*Bacillus polymyxa*, *Rhizobium leguminosarum* and *Pseudomonas fluorescens*) has potential to promote root formation in *Ficus* species cuttings in mass propagation. It seems that the stimulation of rooting and root growth by PGPR can be correlated to production of indole-3-acetic acid (IAA) by the bacteria. Therefore, these results can also be important for the use of these PGPR to multiply organic nursery materials because the use of all formulations of synthetic plant growth regulators, such as indole-3-butyric acid (IBA), is prohibited in organic agriculture throughout the world. A further topic to analyze is whether the cuttings produce auxins themselves after PGPR treatments.

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