The effect of Plant Growth Promoting Rhizobacteria (PGPR) on rooting and root growth of tea (*Camellia sinensis* var. *Sinensis*) cuttings

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Abstract

An attempt was made to induce rooting from cuttings of *Camellia sinensis* by 2000 ppm IBA and PGPR treatments under controlled conditions. The cuttings were prepared from three commercial tea clones, namely Pazar-20, Derepazari-7 and Tuglali-10 in both 2006 and 2007 years. The cuttings were sampled in July and treated with seven bacteria such as *Bacillus* RC23, *Paenibacillus polymyxa* RC05, *Bacillus subtilis* OSU142*, Bacillus* RC03, *Comamonas acidovorans* RC41, *Bacillus megaterium* RC01 and *Bacillus simplex* RC19 and 2000 ppm IBA. *In vitro* production of indole-3-acetic acid (IAA) by PGPR was also determined. The all bacteria showed indole-3-acetic acid (IAA) producing capacity and higher rooting percentages than control treatment. The highest rooting ratio was obtained from 2000 ppm IBA treatments in all clones. Among clones average the highest rooting were observed in Pazar-20 (67.90-70.32%), followed by Derepazari-7 (53.77-54.62%) and Tuglali-10 (22.73-28.58%), respectively. *Bacillus simplex* RC19 and *Paenibacillus polymyxa* RC05 were more effective on rooting for Pazar-20 clone (76.06-76.50%), *Paenibacillus polymyxa* RC05 and *Comamonas acidovorans* RC41 for Derepazari-7 clone (57.20-63.00%) and *Paenibacillus polymyxa* RC05 and *Bacillus megaterium* RC01 treatments for Tuglali-10 clone (40.10-44.09%) in both years.

Keywords: Auxin**,** PGPR, Rooting, Single node cuttings, Tea.

Abbreviations: IAA-indole-3-acetic acid; IBA-indole-3-butyric acid; PGPR-Plant Growth Promoting Bacteria.

Introduction

Camellia sinensis (L.) O. Kuntze. (Family: Theaceae) commonly known as tea plant is one of the most important crops in the world. Although tea plants were first introduced to Turkey in 1920s, the first plantations were established in Black Sea region during 1940s. Tea industry in Turkey has been developed very quickly and now Turkey is important tea producer country ranking 6th place in the world. Turkey is also a few tea producer countries that do not use pesticides in its tea plantations [1, 2].

Tea is an economically valuable plant for the black sea region in Turkey and a means of subsistence for more than 200.000 farmers. The main tea producing area in Turkey is Rize region and 95% of the agricultural fields in Rize are allocated exclusively to the tea cultivation [3].

In Turkey most of the tea plantations was established by using seeds and continuous seed propagation has produced populations with different yield and quality properties reflecting a wide genetic variation. More recently clonal selection studies was conducted in Rize region by Ataturk Tea and Horticultural Research Institute in Rize and several promising tea clones such as Tuglali-10, Derepazari-7 and Pazar-20 have been released [4].

The use of plant growth regulators in vegetative propagation studies in tea plants in Turkey is not common [3]. Previous research has shown that tea cuttings are characterized genotype dependent variable rooting ability [5,6,7,8].

Bench heating, type of cutting, mist, temperature control, rooting media etc. affects the adventitious root formation in tea cuttings [9,10,11]. Auxin has been also known to be intimately involved in the process of adventitious root formation in cuttings [12].

Recent studies confirm that the treatments of seeds or cuttings with non-pathogen PGPR (Plant Growth Promoting Bacteria) such as *Agrobacterium, Bacillus, Streptomyces, Pseudomonas, Alcaligenes* etc. induced root formation in some plants [13,14,15,16]. Although, the mechanisms are not completely clarified, root induction could be result of production of phytohormones such as auxins, cytokinins and gibberellins by bacteria [17]. Consideration the numerous interactions that exist between the different hormones signaling pathways in plants, it is difficult to assess which of these pathways is the primary target of PGPR. More likely, the PGPR alters not just a single, but several, hormonal pathways, which could account for the different morphological changes observed, for example, lateral root elongation and root hair development. One of the more characteristic effects of PGPR is an increased elongation rate, and perhaps initiation rate, of lateral roots resulting in more branched root system architecture [18,19].

The aim of this present study to determine the effects of IBA and PGPR strains, *Bacillus* RC23, *Paenibacillus polymyxa* RC05, *Bacillus subtilis* OSU 142*, Bacillus* RC03, *Comamonas acidovorans* RC41, *Bacillus megaterium* RC01 and *Bacillus simplex* RC19 on rooting and root growth in tea cuttings. *In vitro* production of indole-3-acetic acid (IAA) by PGPR was also evaluated.

Materials and methods

Collection of plant material

Freshly growing tea twigs (~25 cm long) from Pazar-20, Derepazari-7 and Tuglali-10 clones belongs to *Camellia sinensis* var. *sinensis* were collected from tea collection orchard of Ataturk Tea and Horticulture Research Institute, Rize-Turkey in both 2006 and 2007 years. The plants were hard pruned in February so that they developed healthy annual shoots. Previously it was reported that the best cutting collection time to obtain the highest rooting percentage in tea was July and the best IBA treatments were 2000 ppm in Rize region [5,6,20]. Therefore the twigs sampled in July in both years and brought to the laboratory shortly. Twigs were taken from the middle portions of shoots and prepared 7-8 cm long including one or two node with single leaf. The prepared cuttings were used for rooting experiments.

Bacterial strains, isolation, identification and treatments

The information about PGPR used in this study is given in Table 1. The seven non-pathogenic bacteria, *Bacillus* RC23, *Paenibacillus polymyxa* RC05, *Bacillus subtilis* OSU 142*, Bacillus* RC03, *Comamonas acidovorans* RC41, *Bacillus megaterium* RC01 and *Bacillus simplex* RC19 were initially isolated from the rhizosphere of wild raspberry, wheat and tomato. The bacteria were identified based on their whole-cell fatty acid methyl ester (FAMEs) analysis [21] using the MIDI system (Sherlock Microbial Identification System version 4.5, MIDI, Inc., Newark, DE). Bacteria were grown on Nutrient Agar (NA) for routine use, and maintained in Nutrient Broth (NB) with 15% glycerol at –80ºC for long-term storage. For each experiment, a single colony was transferred to 500 ml flasks containing NB, and grown aerobically in flasks on a rotating shaker (150 rpm) for 48 h at 27ºC (Merck KGaA, Germany). The bacterial suspension was then diluted in sterile distilled water to a final concentration of 10^9 CFU ml^{-1}, and the resulting suspensions used to treat tea cuttings.

Table 1. The bacterial strains, codes and its sources

Bacterial strains	Code	Characteristics	Sources
Comamonas acidovorans RC41	RC41	IAA producing capacity	Wild raspberry
Paenibacillus polymyxa RC05	RC05	N_2 fixing + IAA producing capacity	Wheat
Bacillus RC23	RC23	IAA producing capacity	Wild raspberry
Bacillus simplex RC19	RC19	IAA producing capacity	Wild raspberry
Bacillus RC03	RC03	IAA producing capacity	Wheat
Bacillus megaterium RC01	RC01	IAA producing capacity	Wheat
Bacillus subtilis OSU142	OSU142	N_2 fixing + IAA producing	Tomato

		capacity	

Bacterial treatments were performed by dipping the cuttings into the bacterial suspension prepared in sterile water at the concentration of 10^9 cfu ml^{-1} for 30 min. For IBA treatments, the basal portion of cuttings was dipped in an aqueous solution of 2000 ppm IBA (50% ethanol) for 5 min, and allowed to air dry. Cuttings in the control group were treated with sterile water. Following treatments, cuttings were placed in trays filled with perlite media to a depth of 10 cm under mist (15 s/6 min) in a greenhouse maintained at 21 ± 2 ^{0}C. The data on rooting and root growth were obtained after 3 months.

Quantification of IAA production of bacteria

The bacteria were also tested for indole-3-acetic acid (IAA) production, using the method of Bent et al [22]. The flasks were incubated for 18 h at 27°C with 100 rpm rotary shaking. Following this, 125 ml flasks containing 40 mL half-strength TSB, supplemented with 0, 0.1, and 25 mg tryptophan ml^{-1} were each inoculated with 1ml of each strain. After incubation for 48, 72, and 168 h, the density of each culture was measured spectrophotometrically at 600 nm, and then the bacterial cells were removed from the culture medium by centrifugation. The level of indoles present in the culture fluid was estimated colorimetrically. The concentration of IAA in the bacterial eluates was measured by using Salkowski's reagent (50mL 35% $HClO_4$ + 1mL $FeCl_3$). Each reaction mixture was centrifuged. The absorbance at 530 nm in a Shimadzu Spectrophotometer UV-1208 was measured. Bacterial cells were separated from the supernatant by centrifugation at 10,000 rpm for 30 min. The concentration of IAA in each culture medium was determined by comparison with a standard curve. The IAA produced by each strain was measured in triplicate. Besides, after 48, 72, and 168 h of growth, samples were taken for determination of IAA by thin-layer chromatography (TLC) and high performance liquid chromatography–mass spectrometry (HPLC-MS) analysis. Separation of indole in ethyl-acetate fraction was carried out in chloroform-ethyl acetate-formic acid.

Data Analysis

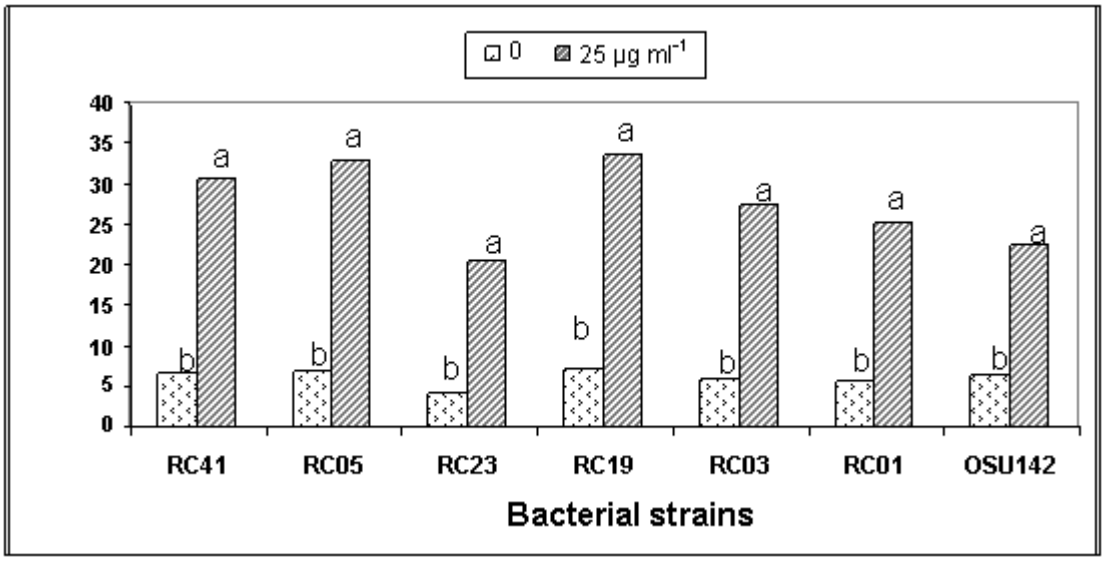
The experimental design used was a randomized complete block with 4 replications. Each replication contained 15 cuttings spaced 50 mm apart. Data were transformed (arcsine) prior to statistical analysis and subjected to analysis of variance using ANOVA and means were separated by Duncan's multiple range tests.

Results and discussion

IAA production of bacteria

All inoculated strains of PGPR were able to produce plant growth–promoting phytohormone, IAA (indole-3-acetic acid) (Figure 1). The amount of IAA produced varied among the bacteria, ranging from 4.3 (*Bacillus* RC23) to 7.2 µg (*Bacillus simplex* RC19) in the absence of tryptophan supplements. However, when seven strains were grown in the presence of 25 µg of tryptophan per ml for approximately 48–168 h, the tested PGPR responded by producing higher levels of IAA. *Bacillus simplex* RC19 and *Paenibacillus polymyxa* RC05 produced higher levels of IAA (33.6 and 32.8 $\mu g ml^{-1}$ $(OD_{600}$ unit$)^{-1}$), while the lowest IAA production was detected from *Bacillus* RC23 (20.4 $\mu g ml^{-1}$ $(OD_{600}$ unit$)^{-1}$) (Table 1).

IAA production



Data were means of three replicates IAA production in average 48, 72, and 168 h pure cultures.

Figure 1. The production of IAA by PGPR in the presence of various concentrations of tryptophan

Rooting and root growth of single node cuttings

The data obtained on the number of main roots per cutting, the highest root length, root dry weight, root quality and rooting percentage for each treatment in tea cuttings are shown in Table 2 and Table 3.

Great variation were observed on all parameters among the IBA, PGPR and control tested in both years ($p<0.01$) (Table 2 and 3).

Table 2. The effect of bacteria on rooting and root growth of cuttings of three tea clones (2006 year)

Clones	*Treatments*	*The highest root length (cm)*	*The number of roots per cutting*	*Root quality (1-4 scale)*	*Rooting (%)*	*Root dry weight (mg)*
Pazar-20	Control	8.65cd	11.14cd	1.25c	33.70c	19.56cd
	IBA 2000 ppm	13.83ab	17.86ab	3.49a	86.40a	38.74a
	RC41	9.10cd	11.59c	3.43a	71.55ab	21.25cd
	RC05	6.89e	10.95cd	2.79ab	74.72ab	19.76cd
	RC23	10.49c	15.35b	2.70ab	67.51bc	20.36cd
	RC19	14.40a	18.53a	2.90ab	76.50a	22.06c
	RC03	8.18d	14.88bc	2.68b	66.66bc	30.29b
	RC01	7.42de	7.73d	2.92ab	65.18bc	16.08d
	OSU142	12.83b	14.97bc	2.71ab	68.89b	16.45cd
	Average	**10.20A**	**13.67A**	**2.76A**	**67.90A**	**22.73A**
Derepazari-7	Control	8.54ab	12.74cd	2.11b	40.12d	18.49c
	IBA 2000 ppm	9.43a	19.23ab	3.18a	75.00a	21.68bc
	RC41	8.48ab	19.09ab	2.67ab	59.00ab	23.97b
	RC05	7.56ab	12.90c	3.10a	63.00a	19.61bc
	RC23	8.17ab	16.97b	2.95ab	48.20c	21.37bc
	RC19	7.50ab	10.80cd	2.70ab	55.28b	24.81ab
	RC03	6.27b	10.17d	2.90ab	62.56a	23.13bc
	RC01	8.37ab	18.34ab	2.80ab	44.54cd	27.00a
	OSU142	9.30a	20.16a	2.66ab	43.92cd	25.36ab
	Average	**8.18B**	**15.60B**	**2.79A**	**54.62B**	**22.82B**
	Control	2.41ab	2.60b	1.25ab	16.39bc	7.00ab

Tuglali-10	IBA 2000 ppm	5.73a	7.55a	3.04a	35.33a	10.98a
	RC41	3.80ab	4.40cd	1.95ab	26.44ab	9.12ab
	RC05	5.60a	7.47a	2.41a	29.74a	10.10a
	RC23	4.60ab	3.46c	1.56ab	18.71b	8.22ab
	RC19	4.31ab	5.73bc	1.70ab	25.87ab	7.52ab
	RC03	3.94ab	4.18bc	1.60ab	20.14ab	7.50b
	RC01	4.42ab	5.40bc	2.20ab	28.00a	8.34ab
	OSU142	2.00b	1.95d	1.00b	4.02c	2.43c
	Average	**4.09C**	**4.75C**	**1.86B**	**22.73C**	**7.91C**

*Values in the same column with different lower-case letters in same clone are significantly different at $P<0.01$.

In 2006, in terms of induction of roots, the different treatments exhibited varying degrees of response within and between clones. The overall, 2000 ppm IBA and PGPR treated cuttings of tea generally had significantly higher numbers of main roots, root length, root dry weight, root quality and rooting percentage than water-treated control cuttings (Table 2).

There were significant differences among clones in terms of higher numbers of main roots, root length, root dry weight, root quality and rooting percentage ($p<0.01$) (Table 2). In 2006 year, the overall, Pazar-20 clone had average 67.90% rooting percentage, and followed by Derepazari-7 clone (54.62%) and Tuglali-10 clone (22.73%) (Table 2).

Table 3. The effect of bacteria on rooting and root growth of cuttings of three tea clones (2007 year)

Clones	*Treatments*	*The highest root length (cm)*	*The number of roots per cutting*	*Root quality (1-4 scale)*	*Rooting (%)*	*Root dry weight (mg)*
Pazar-20	Control	9.40bc	9.97c	1.35c	34.27c	9.30f
	IBA 2000 ppm	16.29a	20.04a	2.84ab	88.35a	26.74ab
	RC41	9.19bc	15.46ab	3.06ab	70.80bc	20.35d
	RC05	7.74c	11.86bc	2.76ab	76.06b	18.71de
	RC23	13.27ab	19.03a	3.26a	76.00b	25.10b
	RC19	15.38ab	14.77b	2.60b	75.09b	23.58c
	RC03	10.08b	15.15ab	2.98ab	73.83bc	27.63a
	RC01	9.30bc	11.95bc	2.67ab	67.23c	17.23e
	OSU142	14.55ab	15.35ab	2.90ab	71.23bc	26.59ab
	Average	**11.69A**	**14.84B**	**2.71A**	**70.32A**	**21.69B**
Derepazari-7	Control	8.32ab	10.60c	2.05b	44.30c	18.04c
	IBA 2000 ppm	11.64a	22.14a	3.08a	68.83a	24.88ab
	RC41	11.24a	17.12ab	2.60ab	57.20b	25.73a
	RC05	7.77ab	12.32bc	2.73ab	52.00bc	22.63ab
	RC23	8.39ab	21.66a	2.40ab	52.00bc	21.86b
	RC19	7.69ab	20.30ab	2.61ab	53.26bc	23.04ab
	RC03	7.52b	13.60bc	2.20ab	54.20bc	23.80ab
	RC01	8.31ab	18.31ab	3.12a	55.30bc	25.00ab
	OSU142	8.41ab	14.98b	2.24ab	46.80bc	23.94ab
	Average	**8.81B**	**16.78A**	**2.56B**	**53.77B**	**23.21A**
Tuglali-10	Control	2.02de	2.54d	1.56ab	26.00d	6.14c
	IBA 2000 ppm	4.38b	6.06a	2.94a	51.14a	12.81a
	RC41	2.12d	3.79c	1.90ab	24.10de	9.84bc
	RC05	4.53ab	5.87a	2.81a	37.30c	11.61b
	RC23	4.31b	4.60bc	1.65ab	22.76de	10.76bc
	RC19	4.51ab	4.90b	1.60ab	19.66e	11.18bc

RC03	3.39c	3.60cd	1.70ab	24.53de	8.12bc
RC01	4.89a	5.30ab	2.08ab	44.09b	10.66bc
OSU142	1.75e	1.90e	1.05b	7.66f	3.65d
Average	**3.54C**	**4.28C**	**1.92C**	**28.58C**	**9.42C**

*Values in the same column with different lower-case letters in same clone are significantly different at $P<0.01$.

Among clones used, the highest rooting percentage for Pazar-20 clone were found as 86.40% in 2000 ppm IBA treatment and followed by 76.50% in *Bacillus simplex* RC19 treatment. In Derepazari-7 clone the highest rooting percentage were observed by 2000 ppm IBA treatment (75.00%) followed by *Paenibacillus polymyxa* RC05 treatment (63.00%). 2000 ppm IBA treatment had also gave the highest rooting percentage (35.33%) in Tuglali-10 clone and followed by *Paenibacillus polymyxa* RC05 treatment (29.74%) (Table 2). The control cuttings of Pazar-20, Derepazari-7 and Tuglali-10 clones exhibited 33.70%, 40.12% and 16.39% rooting percentage indicated lower value than 2000 ppm IBA and all PGPR treatments. As mentioned before *Bacillus simplex* RC19 and *Paenibacillus polymyxa* RC05 produced higher levels of IAA (Figure 1).

The bacteria in general were able to increase the number of roots per cutting and root dry weight. In Pazar-20, Derepazari-7 and Tuglali-10 clones, the highest number of roots per cutting were obtained from *Bacillus simplex* RC19 (18.53), *Bacillus subtilis* OSU142 (20.16) and 2000 ppm IBA treatment (7.55), respectively. These values were 11.14, 12.74 and 2.60 in control cuttings of Pazar-20, Derepazari-7 and Tuglali-10 clones (Table 2).

In 2007, similarly to 2006 overall the highest rooting percentage were observed in Pazar-20 clone (70.32%) and followed by Derepazari-7 (53.77%) and Tuglali-10 clone (28.58%). There were significant differences among IBA, PGPR and control treatments within clone as well ($p<0.01$) (Table 3). The control cuttings of Pazar-20, Derepazari-7 and Tuglali-10 revealed 34.27%, 44.30% and 26.00% rooting percentage. In all clones, 200 ppm IBA resulted the highest rooting percentage (88.35% for Pazar-20; 68.83% for Derepazari-7 and 51.14% for Tuglali-10 clones, respectively). In Pazar-20 and Derepazari-7 clones all PGPR treatments enhanced rooting percentage (67.23-76.06 for Pazar-20 clone and 46.80-57.20% in Derepazari-7 clone, respectively). However the clone Tuglali-10 exhibited different respond to PGPR treatments. Only *Paenibacillus polymyxa* RC05 (37.30%) and *Bacillus megaterium* RC01 (44.09%) increased rooting percentage compared to control (Table 3).

The IBA and PGPR treatments was also resulted higher root length, the number of roots per cutting, root quality and root dry weight particularly in Pazar-20 and Derepazari-7 clones (Table 3).

The overall rooting ratio of tea clones (averages of 2006 and 2007 years) were the highest as 69.11% in Pazar-20 clone and followed by Derepazari-7 (54.20%) and Tuglali-10 (25.66%), respectively (Figure 2).

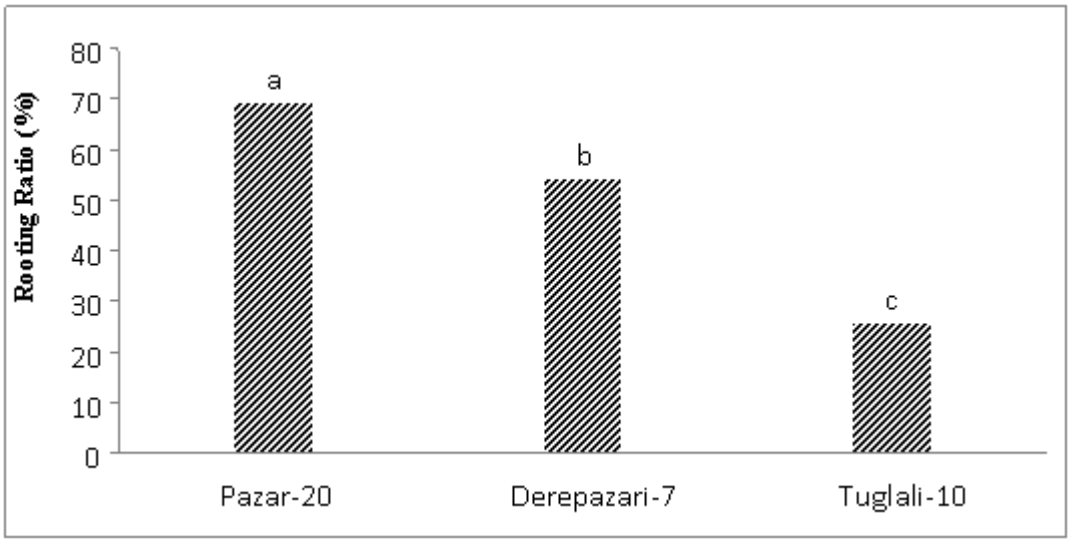


Figure 2. The overall rooting ratio of tea clones (averages of 2006 and 2007 years)

Many micro organisms that interact with plants can synthesize hormones similar to those produced by the plant as growth regulator, such as auxins, gibberellins and cytokines [23]. Of these, auxin is one of the most well-known hormones because of its important roles in the initial processes of lateral and adventitious root formation [24] and root elongation [25]. As shown in Table 2, PGPR produce auxin themselves and may also

enhance plant synthesis of auxin [26]. We obtained better rooting and root growth from IBA treatment and PGPR inoculations compared to control. The rooting ratio of PGPR was found to 2000 ppm IBA treatments. Among PGPR the highest rooting percentages and root growth were observed in higher IAA produced PGPR such as *Bacillus simplex* RC19 and *Paenibacillus polymyxa* RC05. IAA is the most commonly produced auxin in nature, synthesized mainly through tryptophan dependent pathways. The endogenous level of IAA in the plant is also important for successful rooting. Furthermore, other indolic compounds, such as indole-pyruvic, indole-acetamide and indole-carboxylic-acid, can be involved in root formation [27].

These results show that the treatment of IAA producer PGPR is useful for root induction in tea cuttings. It is evident that the treatment of PGPR on cuttings of different plant species responded rooting and root growth increase [28,29]. Therefore, these results could be important for woody plants to obtain higher rooting percentage by inoculation with bacteria, in particular organic growing conditions. McAfee et al. [30] showed that rooting of *Pinus* was higher when they were inoculated with bacteria strains. Ercisli et al. [15] and Esitken et al. [16] tested PGPR for rooting in kiwifruit and sour cherry cuttings and found that PGPR were effective to obtain high rooting percentages. Our results support the findings of Ercan et al. [31] who demonstrated that the root number were increased in Madder (*Rubia tinctorum*) after PGPR inoculation. Caesar and Burn [32] also observed that seedlings of apple gave better lateral root when treated with PGPR.

As conclusion, this study demonstrating that the higher IAA producing PGPR, particularly *Bacillus simplex* RC19, *Paenibacillus polymyxa* RC05, *Bacillus megaterium* RC01 and *Comamonas acidovorans* RC41 had potential to root formation in tea cuttings in the mass clonal propagation. The stimulation of rooting and root growth by PGPR is could be result of production of indole-3-acetic acid by the bacteria. These results were also important for use of these PGPR to multiply organic nursery materials.

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