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Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (*Actinidia deliciosa*) stem cuttings

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ABSTRACT

The effects of plant growth promoting rhizobacteria (PGPR) on the rooting and root growth of semi-hardwood and hardwood kiwifruit stem cuttings were investigated. The PGPR used were *Bacillus* RC23, *Paenibacillus polymyxa* RC05, *Bacillus subtilis* OSU142, *Bacillus* RC03, *Comamonas acidovorans* RC41, *Bacillus megaterium* RC01 and *Bacillus simplex* RC19. All the bacteria showed indole-3-acetic acid (IAA) producing capacity. Among the PGPR used, the highest rooting ratios were obtained at 47.50% for semi-hardwood stem cuttings from *Bacillus* RC03 and *Bacillus simplex* RC19 treatments and 42.50% for hardwood stem cuttings from *Bacillus* RC03. As well, *Comamonas acidovorans* RC41 inoculations indicated higher value than control treatments. The results suggest that these PGPR can be used in organic nursery material production and point to the feasibility of synthetic auxin (IBA) replacement by organic management based on PGPR.

Key terms: PGPR, stem cuttings, kiwifruit, rooting, auxin

INTRODUCTION

Kiwifruit are utilized almost exclusively as fresh fruits, with the exception of a small proportion juiced and mixed with other juices and used in wine coolers. The plant also has an ornamental value (1).

Kiwifruit was first introduced to Turkey in the 1980s. In the last few years significant progress has been achieved. Several experiments have been conducted in different agro-ecological areas; mainly in the Black Sea, Marmara, Aegean and Mediterranean regions, and considerable information has been collected on plant-climate and plant-soil relations and the area for kiwifruit cultivation in Turkey is expanding (2). Interest in self-rooting kiwifruit plants has increased, especially in some regions of Turkey where winter-damaged trunks can be replaced more rapidly from adventitious shoots. The demand for these plants has led nursery operators to look for effective means of propagating large number of plants rapidly. More recently, organic kiwifruit production has also gained importance in Turkey and there is increased interest among farmers in nursery materials for organically producued kiwifruit.

Kiwifruit may be propagated by various methods, such as grafting seedlings, stem cuttings, root cuttings and tissue culture (3). The most rapid and inexpensive production method of kiwifruit would be of considerable commercial value. Growing cuttings on their own roots could achieve this purpose by eliminating the need for producing rootstocks. Previous research has shown that kiwifruit stem cuttings are characterized by a variable rooting ability (4, 5, 6). Bench heating, mist, temperature control, growth substance

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treatments are always required to obtain satisfactory propagation in kiwifruit stem cuttings, making it one of the most difficult species to root (7, 8). In addition, the type of cutting and the collecting time have been reported to strongly influence rooting of kiwifruit cuttings (9, 10).

PGPR (plant growth promoting bacteria) have gained world wide importance and acceptance. The mechanisms involved in plant growth promotion by PGPR involve direct and indirect effects. Direct effects occur when PGPR produces substances such as phytohormones. These microorganisms are the potential tools for sustainable agriculture and the trend for future (11, 12, 13, 14). Recent studies confirm that the treatments of seeds or cuttings with non-pathogen bacteria, such as *Agrobacterium, Bacillus, Streptomyces, Pseudomonas, Alcaligenes* etc. induced root formation in some plants because of natural auxin production of bacteria (14, 15, 16, 17, 18). Although, the mechanisms are not completely understood, root induction by PGPR is the accepted result of phytohormones, such as auxin production, inhibition of ethylene synthesis and mineralization of nutrients by PGPR (18, 19). Considering the numerous interactions between the different hormonal signaling pathways in plants, it is difficult to assess which of these pathways is the primary target of PGPR. More likely, PGPR alters several, hormonal pathways. This 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 (20, 21).

Environmentally friendly applications in agriculture have gained more importance, in particular in horticulture and nursery production. The use of PGPR for nursery material multiplication may be important for obtaining organic nursery material 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.

The aim of this study was to determine the effects of 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 kiwifruit cv. Hayward semi-hardwood and hardwood stem cuttings. *In vitro* production of indole-3-acetic acid (IAA) by PGPR was also evaluated.

MATERIALS AND METHODS

Bacterial strains, isolation and identification of bacteria

The PGPR used in this study are 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 in late summer from the rhizosphere of wild raspberry, wheat and tomato plants in northeastern Turkey, except for *Bacillus subtilis* OSU 142 from Ohio, USA. The isolation of bacteria was carried out according to Ramos Solano et al. (22). The bacteria were identified based on their whole-cell fatty acid methyl ester (FAMEs) analysis (23) 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 kiwifruit cuttings.

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. (24). 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.

Plant materials

Semi hardwood and hardwood stem cuttings of kiwifruit cv. Hayward were prepared from middle parts of vigorous shoots of 14-yr-old plants during June and January in both 2006 and 2007. The cuttings had three buds and were 20 cm in height and 1 cm in diameter. 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. Cuttings in the control group were treated with sterile water. For IBA treatments, the basal portion of cuttings was dipped in an aqueous solution of either 2000 or 4000 ppm IBA (50% ethanol) for 5 min, and allowed to air dry. 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 were obtained after 3 months. The evaluated parameters determined by Orhan et al. (25) were rooting percentage, the number of main roots per cutting, the highest root length (cm), average root diameter (mm), root dry weight per cutting (mg) and root quality (1-5 scale).

Data Analysis

The experimental design used was a randomized complete block with 4 replications. Each replication contained 10 cuttings spaced 50 mm apart. Data were subjected to analysis of variance using ANOVA and means were separated by Duncan's multiple range tests ($p<0.05$). There were no statistical differences between years, therefore the data were pooled.

TABLE 1

The bacterial strains, codes and sources

Bacterial strains	Code	Characteristics	Sources
Bacillus RC23	RC23	IAA producing capacity	Wild raspberry
Paenibacillus polymyxa RC05	RC05	N_2 fixing + IAA producing capacity	Wheat
Bacillus subtilis	OSU 142	N_2 fixing + IAA producing capacity	Tomato
Bacillus RC03	RC03	IAA producing capacity	Wheat
Comamonas acidovorans RC41	RC41	IAA producing capacity	Wild raspberry
Bacillus megaterium RC01	RC01	IAA producing capacity	Wheat
Bacillus simplex RC19	RC19	IAA producing capacity	Wild raspberry

RESULTS AND DISCUSSION

IAA production of bacteria

Great variation was observed in the IAA production capacity among PGPR tested ($p<0.05$) (Table 2). All inoculated strains of PGPR were able to produce plant growth-promoting phytohormone, IAA (indole-3-acetic acid) (Table 2), affirming the natural ability of PGPR in synthesizing IAA. 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 μgml^{-1} $(OD_{600}$ unit$)^{-1}$), while the lowest IAA production was detected from *Bacillus* RC23 (20.4 μgml^{-1} $(OD_{600}$ unit$)^{-1}$) (Table 2). 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 (26). Among them, auxin is one of the most well-known phytohormones because of its important role in the initial processes of lateral and adventitious root formation (27) and root elongation (28). Our results showed that PGPR produce auxin themselves and may also affect auxin synthesis of cuttings (29). 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 (30).

Rooting and root growth of stem cuttings

The data obtained on the number of main roots per cutting, the highest root length, average root diameter, root dry weight, root quality and rooting percentage for each treatment in kiwifruit semi-hardwood and hardwood stem cuttings are shown in Table 3 and Table 4.

Great variation on all parameters was observed among the PGPR tested in both semi-hardwood and hardwood stem cuttings types ($p<0.05$) (Table 3 and 4).

In semi-hardwood cuttings, in terms of induction of roots, the different treatments exhibited varying degrees of response. The PGPR treated semi-hardwood stem cuttings of kiwifruit generally had significantly higher numbers of main roots, root length, root diameter, root dry weight, root quality and rooting percentage than water-treated

TABLE 2

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

Bacterial strains	IAA production ($\mu g\ ml^{-1}$ $(OD_{600}$ unit$)^{-1}$)	
	Control	Tryptophan (25$\mu g\ ml^{-1}$)
RC23	4.3±0.7b	20.4±1.6c
RC05	6.8±0.9ab	32.8±2.6a
OSU 142	6.3±0.8ab	22.4±2.1bc
RC03	5.9±0.6ab	27.3±2.7b
RC41	6.7±0.5ab	30.5±2.4ab
RC01	5.6±0.5ab	25.3±1.7bc
RC19	7.2±0.5a	33.6±2.6a

* Values in the same column with different lower-case letters in same clone are significantly different at $p<0.05$. Average ± standard error from three separate experiments. Data were means of three replicates IAA production in average 48, 72, and 168 h pure cultures.

control stem cuttings. However, in general these parameters were lower than 2000 ppm and 4000 ppm IBA (indole-3-butyric acid) treatments.

The highest number of main roots per cutting (5.18) was obtained using 4000 ppm IBA followed by 2000 ppm IBA (4.89), *Bacillus* RC03 (3.40) and *Paenibacillus polymyxa* RC05 treatments (3.36) (Table 3).

The highest root length and diameter of stem cuttings treated with IBA and bacteria ranged from 9.76 cm (IBA 4000 ppm), 9.22 cm (IBA 2000 ppm), 8.63 cm (*Bacillus simplex* RC19) and 7.70 cm (*Comamonas acidovorans* RC41), respectively (Table 3).

The best root dry weight (mg) was observed in the 4000 ppm IBA treatment as 22.33 mg, while the lowest in control cutting was 2.56 cm (Table 3).

The root quality was the highest in the 4000 ppm IBA treatment (3.33), followed by the 2000 ppm IBA treatment (3.02) and *Paenibacillus polymyxa* RC05 (2.82). The root quality in control cuttings were the lowest among the treatments (1.63) (Table 3).

The differences in rooting with different treatments were significant, with the highest rooting percentage observed in 4000 ppm IBA, followed by 2000 ppm IBA (57.50%), and equally 47.50% in *Bacillus* RC03 and *Bacillus simplex* RC19, respectively. The control treatment gave the lowest rooting percentage (12.50%) (Table 3).

In hardwood stem cuttings, 4000 ppm IBA gave the highest number of main roots (4.74), the greatest root length (9.11 cm), root diameter (1.34 mm), root dry weight (22.11 mg), root quality (2.87) and rooting percentage (65.00%), respectively (Table 4).

Increases in rooting percentage and root growth of cuttings varied according to PGPR tested. All PGPR increased root growth and rooting percentage over the control. Among PGPR treatments, *Bacillus* RC03 were found more effective on the number of main root (3.53) and rooting (42.50%), *Bacillus simplex* RC19 on the highest root length (7.27 cm), *Bacillus* RC23 on root diameter (1.22 mm), *Paenibacillus polymyxa* RC05 on root dry weight (19.32 mg) and root quality (2.44) and *Comamonas acidovorans* RC41 on rooting (42.50%), respectively. Control cuttings gave 0.00% rooting percentage (Table 4).

These results show that the PGPR treatment is useful for root induction in kiwifruit stem cuttings. Auxin production by bacteria is one of the explanation of

TABLE 3

The effect of bacteria on rooting and root growth of semi-hardwood cuttings of kiwifruit cv. Hayward (Average of 2006 and 2007 years)

Treatments	The number of main roots per cutting	The highest root length (cm)	Average root diameter (mm)	Root dry weight per cutting (mg)	Root quality (1-5 scale)	Rooting (%)
Control	0.53f	0.70f	0.27e	2.56g	1.63e	12.50g
IBA 2000 ppm	4.89b	9.22ab	1.18b	19.63b	3.02b	57.50b
IBA 4000 ppm	5.18a	9.76a	1.30a	22.33a	3.33a	72.50a
RC23	2.67d	6.73cd	1.05c	13.89e	2.14d	32.50e
RC05	3.36c	6.17de	1.12bc	15.37d	2.82bc	40.00d
OSU142	2.47de	5.70de	0.92d	12.47f	2.40cd	25.00f
RC03	3.40c	6.40d	1.19b	17.18c	2.53c	47.50c
RC41	2.88cd	7.70c	1.03c	15.76d	1.70e	40.00d
RC01	2.47de	7.60cd	0.90d	14.86de	2.12d	32.50e
RC19	3.07d	8.63ab	1.13bc	17.29c	2.00de	47.50c
LSD	0.25	1.24	0.11	1.02	0.24	4.67

* Values in the same column with different lower-case letters are significantly different at $p<0.05$.

root induction in kiwifruit stem cuttings. Another explanation could be that the cuttings can produce auxin themselves after PGPR inoculation. Unfortunately we did not analyze auxin levels in cuttings during the experiment. The PGPR produced higher percentages of root growth and better quality in terms of root length, diameter, root dry weight etc (Table 3 and Table 4). PGPR is able to exert a beneficial effect on plant growth such as increases in root growth and weight (31). It is evident that the treatment of PGPR on cuttings of different plant species showed genotype dependent rooting and increased root growth (32, 33). Patten and Glick (34) reported that IAA producing *Pseudomonas putida* increased the length of canola seedling roots on average 35 to 50%. Bae et al. (35) stimulated initial development of adventitious roots in cucumber and rose cuttings, using different PGPR isolates. They determined that cucumber seedling cuttings showed various responses to the isolates tested, which is in accordance with our results. Therefore these results could be important for particularly difficult-to-root woody plants, in our case kiwifruit, in order to obtain higher rooting percentages by inoculation with bacteria. McAfee et al. (36) showed that rooting of *Pinus* was higher when they were inoculated with bacteria strains. Ercisli et al. (37) and Esitken et al. (17) tested PGPR for rooting in rose hip and sour cherry cuttings and found that PGPR were effective to obtain high rooting percentages. Our results support the findings of Ercan et al. (38), who demonstrated that the root numbers increased in Madder (*Rubia tinctorum*) after PGPR inoculation. Caesar and Burn (39) observed that seedlings of apple gave better lateral roots when treated with PGPR. Kaymak et al. (2008) also demonstrated that mint cuttings inoculated with PGPR resulted in higher rooting percentage and root dry weight.

In conclusion, this study demonstrated that the PGPR belonging to genus *Bacillus*, *Paenibacillus* and *Comamonas* has potential to promote root formation in kiwifruit cuttings in mass clonal propagation. It seems that the stimulation of rooting and root growth by PGPR can be correlated to production of indole-3-acetic acid by the bacteria. In our study in general, the PGPR has higher IAA producing

TABLE 4

The effect of bacteria on rooting and root growth of hardwood cuttings of kiwifruit cv. Hayward (Average of 2006 and 2007 years)

Treatments	The number of main roots per cutting	The highest root length (cm)	Average root diameter (mm)	Root dry weight (mg)	Root quality (1-5 scala)	Rooting (%)
Control	0.00g	0.00e	0.00d	0.00g	0.00f	0.00f
IBA 2000 ppm	4.36b	8.23ab	1.28ab	18.14c	2.71ab	50.00b
IBA 4000 ppm	4.74a	9.11a	1.34a	22.11a	2.87a	65.00a
RC23	2.47f	5.63cd	1.22ab	16.25d	2.00d	32.50de
RC05	2.87de	6.17c	1.13b	19.32b	2.44b	35.00d
OSU142	2.67ef	5.33cd	1.09bc	15.02e	2.28c	27.50e
RC03	3.53c	5.03d	1.06bc	18.52bc	2.42b	42.50c
RC41	2.80e	6.93bc	0.95c	14.58ef	1.66e	42.50c
RC01	2.53ef	7.07bc	1.00bc	13.40f	2.30c	35.00d
RC19	3.13d	7.27b	1.02bc	14.57ef	2.20cd	40.00cd
LSD	0.30	1.06	0.14	1.16	0.19	7.10

* Values in the same column with different lower-case letters in same clone are significantly different at $p<0.05$.

capacity, which also resulted in higher rooting percentages in cuttings. Among PGPR used, as mentioned before, *Bacillus* RC03, *Bacillus simplex* RC19 and *Comamonas acidovorans* RC41 were found to be produce more IAA and these bacteria are also more effective on rooting in kiwifruit stem cuttings, maximizing yield of rooted clonal cuttings in nurseries. As is well-known, the use of chemicals in plant propagation can produce environmental problems and may increase the cost of propagation in nurseries. Therefore, these results can also be important for the use of these PGPR to multiply organic nursery materials. In fact, several commercial PGPR products are already being used in organic agriculture practice. A further topic to analyze is whether the cuttings produce auxin themselves after PGPR treatments.

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