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AUXIN-PRODUCING PLANT GROWTH-PROMOTING RHIZOBACTERIA PROMOTE ROOT FORMATION OF *EPIPREMNUM AUREUM* CUTTINGS

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ABSTRACT

This work was conducted to determine the possible influences of auxin-producing plant growth promoting rhizobacteria (PGPR) on root formation of *Epipremnum aureum* stem cuttings for environment friendly commercial production. *Agrobacterium rubi* A-16*, Bacillus megaterium* M-3, TV-91C and TV-6D were used to increase rooting performance. The new root formation was higher (51.67-75.00%) in all bacteria strain treatments as compared to control (36.67%). Root and shoot length were longer in cuttings inoculated with *Agrobacterium rubi* A-16 compared with other bacteria strains. The root and shoot lengths were lower than bacteria treatments in control cuttings. The dry matter content of roots varied from 7.27% (M-3) to 8.33% (A-16). The highest new leaf number (2.97) was counted in TV-6D followed by 2.87 in treatment TV-91C. The leaf area and chlorophyll content varied from 8.41 cm^2 (control) to 10.32 cm^2 (M-3) and 22.60 (TV-6D) to 25.97 (TV-91C), respectively. The mean values of examined properties of *Epipremnum aureum* cuttings treated with auxin-producing plant growth promoting rhizobacteria were better than control. Plus, when bacteria strains were evaluated among themselves, it could be said that the effect of *Agrobacterium rubi* A-16 on the examined parameters were higher than other bacteria strains and can be suggested for producers of *Epipremnum aureum* and other ornamental species for vegetative propagation.

Keywords: *Agrobacterium rubi*, *Bacillus megaterium*, ornamental plants, propagation
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INTRODUCTION

Epipremnum aureum, mostly known as pothos, belongs to family Araceae. Pothos is an important ornamental foliage plant species which is indoor plant, reduce the amount of ozone in the environment to a considerable extent and capable of removing indoor air pollutants such as xylene, formaldehyde and benzene (Greig, 2004; Papinchak *et al.,* 2009; Das *et al.,* 2017). Pothos has a reputation as a traditional anticancer preparation as well as a remedy for skin diseases in some countries such as Malaysia and Singapore (Das *et al.,* 2017).

The commercial production of ornamental foliage crops is commonly made by using cutting propagation method (Blythe *et al.,* 2004). However, many factors that influenced the rooting of cuttings such as genetic, physiological, phenological state of the plants or environmental factors. For example, cell differentiation and new root formation of cuttings are widely affected by genetic factors (Pio *et al*., 2010). Therefore, Oliveira *et al*. (2010) suggested that the external applications with plant growth regulators, such as auxins, may be used for difficult to rooting plant species. Similarly, Blythe *et al.* (2004) reported that auxins such as indole-3-butyric acid (IBA), 1-naphthaleneacetic acid (NAA), or in combination can be used for cutting propagation. However, Sauer *et al.* (2013) reported that the application of the synthetic plant growth regulators at high concentrations may cause undesirable situations such as plant toxicity or inhibitory effect of rooting. Chemicals such as fertilizers, pesticides and plant growth regulators can damage the environment and increased agricultural production prices. On the other hand, plant growth promoting rhizobacteria (PGPR) can contribute to sustainable agriculture, and can be used as bio-stimulant or bio-fertilizer. PGPR affects positively plant growth and yield by increasing the number and length of roots and chlorophyll content of leaves (Mohite, 2013; Ruzzi and Aroca, 2015).

It is known that PGPR influences plant growth directly or indirectly positively. Phytohormones such as auxins and GAs that can be synthesized and secreted by PGPR are directly caused plant growth promoting (Kaymak, 2010; Kaymak, 2019). The auxins produced by PGPR can be used instead of synthetic auxins for success to rooting of cuttings in many plant species. Due to problems in rooting of cuttings, auxin-producing PGPR applications can be recommend as one of the alternative

auxiliary applications used to promote rooting of cuttings of different plant species such as *Ficus benjamina*, *Rosa hybrid*, rosehip, mint and ornamental shrubs (Bredmose *et al.,* 2004; Ercisli *et al.,* 2004; Pacholczak *et al.,* 2005; Siddiqui and Hussain, 2007; Kaymak *et al.,* 2008; Sezen *et al.,* 2014).

Several investigations have been reported on the effect of auxin and other phytohormones on rooting performance of *E. aureum*. For example, Sharma (2013) reported that stem cuttings of *E. aureum* treated with IAA + IBA (Indole-3-acetic acid and Indole-3-butyric acid) significantly affected and promoted the rooting of cuttings. Chemical inputs such as synthetic auxins are used to increase rooting of cuttings, but due to the careless, uncontrolled and repeatedly use for many years, it causes accumulation of toxic chemical wastes and residues in the environment. PGPR is an environmentally friendly and sustainable method to overcome these negative effects at every step of production. However, there is no available detailed report on the effect of auxin-producing plant growth promoting rhizobacteria on root formation of *E. aureum*. Therefore, this work was conducted out to determine the possible influences of inoculation with *Agrobacterium rubi* A-16, *Bacillus megaterium* M-3, TV-91C and TV-6D on root formation of *E. aureum* stem cuttings for environment friendly commercial production.

MATERIALS AND METHODS

This research was conducted under controlled greenhouse conditions at Plant Production, Application and Research Centre of Atatürk University, Erzurum, Turkey, between 25 January 2016 and 26 April 2016. About ten-year old *Epipremnum aureum* of three mother plants that have same development stage were used for preparation of cuttings.

Eye cuttings that have a leaf with a single stem node are widely used in the propagation of pothos (Griffith, 1998). Healthy stems from the basal portion of branches with short internodes were used. Cuttings were soaked in clean water immediately after branches were cut from the mother plants. Cuttings of *E. aureum* were prepared 10 to 15 cm long.

Plant growth promoting rhizobacteria (*Agrobacterium rubi* A-16, *Bacillus megaterium* M-3, TV-91C and TV-6D) used in this research were received from PGPR collections of the Department of Plant Protection, Agriculture Faculty of Atatürk University. Some biochemical characteristics and hypersensitivity test results of *Agrobacterium rubi* A-16, *Bacillus megaterium* M-3, TV-91C and TV-6D were shown in Table 1. Hypersensitivity tests of bacterial strains used in this research were made according to the suggestions of Klement *et al.* (1964).

Table 1. Some biochemical characteristics of *Agrobacterium rubi* A-16, *Bacillus megaterium* M-3, TV-91C and TV-6D.

	A-16	M-3	TV-91C	TV-6D
Isolate Origin	**Apple**	**Rice**	**Wheat**	**Wheat**
Hypersensitivity reaction	-	-	-	-
IAA production	+	+	+	+
ACC Deaminase activity	+	+	+	+
Nitrogen fixation	-	s+	+	+
Phosphate solubilization	+	+	w+	+

IAA: indole-3-acetic acid, ACC: 1-aminocyclopropane-1-carboxylate, S: strongly, W: weakly

Bacteria strains were stored in nutrient broth (NB) selective growth medium amended with 30% glycerol at - 80°C prior to use. A single colony from each stock bacteria cultures were transferred to flasks (500 ml) containing NB. Then, bacteria strains were grown aerobically in flasks on a shaker (rotating 150 rpm) for 24 h at 27C. A UV-visible spectrophotometer (Shimadzu, Japan, UV 1201, SN A1080) was used to check the purity and the optical density of bacterial suspensions. Sterile distilled water was used to dilute the resulting bacterial suspensions and 10^8 cfu/ml was the final concentration of bacterial suspensions. Finally, the suspensions of *Agrobacterium rubi* (A-16), *Bacillus megaterium* (M-3), *Bacillus megaterium* (TV-91C) and *Bacillus megaterium* (TV-6D) were used to treat cuttings. *Epipremnum aureum* cuttings except for control (pure water) were held in bacterial suspensions for 45 min (Kaymak *et al.,* 2008; Sezen *et al.,* 2014). A total of 60 cuttings were used for each treatment. A total of 300 cuttings (replicate including 20 cuttings per replicate) were prepared for experiment using mother plants available. The experimental design of this work was a completely randomized block design with 3 replications.

Epipremnum aureum cuttings were transferred to trays (20 x 50 cm) filled with sterile perlite at a depth of 7-8 cm after bacterial application. Irrigation was made automatically three times a day by using mini sprinkler under 22 ± 2°C (Sezen *et al.* 2014). Rooting percentage (%), root length (cm), dry matter content of roots (%), new leaf number, leaf area (cm^2) and shoot length (cm) were determined at the end of the work (90 days). The leaf greenness of the plants was measured by using a portable SPAD-502 chlorophyll meter (SPAD-502; Konica Minolta Sensing, Inc., Japan) used to estimate the total leaf chlorophyll content.

All data were subjected to Analysis of Variance (ANOVA), Duncan's multiple range test was used to compare the differences between means.

RESULTS AND DISCUSSION

The effect of auxin-producing plant growth promoting rhizobacteria (PGPR) on rooting percentage, root length and dry matter content of roots in *Epipremnum aureum* stem cuttings were statistically significant ($P < 0.05$). Rooting responses of *E. aureum* cuttings to PGPR application is presented in Table 2. The highest rooting percentage was determined in *Agrobacterium rubi* A-16 (75 %), followed by *Bacillus megaterium* M-3 (61.67%), TV-91C (53.33%) and TV-6D (51.67%), while the lowest were observed in control (36.67%). According to these results, it can be clearly stated that the positive effect of *Agrobacterium rubi* A-16 and *Bacillus megaterium* M-3 on rooting percentage is higher than *Bacillus megaterium* TV-91C and TV-6D (Table 2). This is the first study to demonstrate that PGPR can increase rooting percentage, root length, dry matter content of roots of *E. aureum*. Our results indicate that all the bacteria strains were effectively improved rooting percentage and root length of *E. aureum*. Results are similar to those of Kaymak *et al.* (2008) and Sezen *et al.* (2014), which indicated that stem cuttings and semi-hardwood cuttings of mint and *Ficus benjamina* L. rooted best when, treated with with auxin-producing *Pseudomonas putida* (BA-8) and *Bacillus subtilis* (BA-142). Sezen *et al.* (2014) reported that the rooting percentage of *Ficus benjamina* L. was 100% in all auxin-producing *Agrobacterium rubi* A-1 and A-18, *Pseudomonas putida* BA-8, *Bacillus subtilis* BA-142. Similarly, *Bacillus cereus* strain UPMLH24 increased rooting percentage (96%) of black pepper cuttings and this was better than hormonal treatment with 1000 ppm IBA (88% rooting) (Aziz *et al*. 2015).

Root length of *Epipremnum aureum* changed according to the bacterial treatments and significant root length increase was obtained with bacterial treatments as compared with control. As seen on Table 2, the lowest mean value of root length was determined in control (16.50 cm) and the highest value was obtained in *Agrobacterium rubi* A-16 (23.90 cm). The average percentage of root length increase was 45%, 36%, 32% and 28% when A-16, TV-91C, M-3 and TV-6D were applied, respectively. When the bacteria activity was taken into consideration, it was observed that *Agrobacterium rubi* A-16 was more efficacious than the others (Table 2; Fig.1). Mayak *et al.* (1999) declared that roots of mung bean cuttings treated with *Pseudomonas putida* GR12-2 were longer compared with control cuttings. Similar results about root length increase were obtained in lettuce and tomato when treated with *Pseudomonas putida* GR12-2 and *Azosprillum brasilense* FT 326 (Hall *et al.* 1996; Ribaudo *et al.,* 2006). Sezen *et al*. (2014) reported that the root length of *Ficus benjamina* L. cuttings which were inoculated with *Bacillus subtilis* BA-142 and Agrobacterium rubi A-18 was longer than the control. In addition, the effect of some PGPR on root elongation is caused by the effect of auxin on growth promotion (Jackson, 1991; Mayak *et al.,* 1999). Furthermore, ethylene production at the roots of PGPR treated plants is reduced by the ACC-deaminase activity of PGPR and causes root elongation. The obtained results in this work were supported by previous studies (Hall *et al.,* 1996; Mayak *et al.,* 1999; Ribaudo *et al.,* 2006; Sezen *et al.,* 2014).

Figure 1. Effect of auxin-producing plant growth promoting rhizobacteria on root formation of *Epipremnum aureum* stem cuttings.

Bacterial treatments affected the dry matter content of roots significantly ($P< 0.05$). Root dry matter content of *Epipremnum aureum* cuttings inoculated with *Agrobacterium rubi* A-16 (8.33%) was higher than

control and the other bacterial treatments (Table 2). In addition, the lowest mean value of dry matter content of roots was determined in *Bacillus megaterium* M-3 (7.27%) and in control (7.73%). Raasch *et al.,* (2013) declared that fresh and dry weight of the root of Eucalyptus mini-cuttings increased when treated with *Bacillus subtilis*. Similarly, Kaymak *et al.* (2008) reported that bacterial treatments affected the dry matter content of mint roots and inoculated with *Bacillus megatorium* M3 increased the dry matter content according to the control. *Agrobacterium rubi* A-1, *Azosprillum brasilense* FT 326 and *Azosprillum brasilense* Sp7 increased the root fresh weight of pistachio, tomato and photinia when compared to the control (Ribaudo *et al.,* 2006; Orhan *et al*. 2007; Larraburu *et al.,* 2007). Previous works with PGPR studied on different species have been reported confirmative findings supporting our data in the present work.

The data pertaining to new leaf number per cutting and leaf area are presented in Table 3. Different bacteria strains had significant effect on new leaf number per cutting. The results of this study showed that the highest new leaf number (2.97) was determined in *Bacillus megaterium* TV-6D followed by 2.87, 2.73 and 2.30 in treatments *Bacillus megaterium* TV-91C, *Bacillus megaterium* M-3 and *Agrobacterium rubi* A-16 treatments, respectively. The lowest new leaf number (1.57) was recorded in control. On the other hand, it was determined that the differences between bacterial treatments concerning the new leaf number when the bacterial treatments compared to each other were not statistically significant (Table 3).

The mean value of leaf area of *E. aureum* varied depending on treatments and significant leaf area increase was determined with *Bacillus megaterium* M-3 and *Bacillus megaterium* TV-6D treatments as compared with control and *Bacillus megaterium* TV-91C and *Agrobacterium rubi* A-16 (Table 3). Leaf area was ranged from 8.41 cm^2 (control) to 10.32 cm^2 (*Bacillus megaterium* M-3). According to the control, the average percentage of leaf area increase was 23%, when M-3 was applied (Table3).

The effects of bacteria inoculation on shoot length and chlorophyll content of *E. aureum* cuttings were found statistically significant at 0.05 probability levels (Table 4). Shoot length was ranged from 2.93 cm (control) to 5.10 cm (*Agrobacterium rubi A-16*). The bacterial treatments, except for *Bacillus megaterium* TV-6D, significantly increased ($p < 0.05$) the SPAD values (22.73 – 25.97) of *Epipremnum aureum*. Treatment with *Agrobacterium rubi* A-16, *Bacillus megaterium* TV-91C and M-3 deepened the color of the *Epipremnum aureum* leaves, and the SPAD measurement increased 9%–14% compared to the control.

The positive effect of bacterial treatments on new leaf number, leaf area, shoot length and chlorophyll content of *E. aureum* could be explained with the direct effect of PGPR on plant growth. According to the results of recent studies, it is reported that PGPR can influence growth in different ways such as biological nitrogen fixation (BNF), solubilisation of phosphate, synthesized plant hormones like auxins, cytokinins etc. and promoting of vegetative growth is recorded in a large scale of species including mint, *Ficus benjamina* L. and different crops such as sour cherry (Esitken *et al.,* 2003), rosehip (Ercisli *et al.,* 2004), kiwifruit (Ercisli *et al.,* 2003, Erturk *et al.,* 2010). In other words, PGPR can synthesize and produced auxin which is a quantitatively important plant growth regulator and inoculation with auxin-producing PGPR species cause increasing the plant growth and yield (Vessey, 2003). Thus, increased new leaf number, leaf area, shoot length and chlorophyll content of *E. aureum* of inoculated cuttings may be due to bacterial N_2 fixation, higher N accumulation and better root growth that improves more water and nutrient intake. Higher N incorporation was achieved as enzyme and protein formation provided better physiological activity. The higher N also contributed to the production of chlorophyll and as a result, increased photosynthetic activity (Mia *et al.,* 2005; Kaymak *et al.,* 2013).

Comparative cost and benefit analysis of plant growth promoting rhizobacteria use in *E. aureum* production were given in Table 5. The highest net profit was determined in *Agrobacterium rubi* A-16 (305 $), followed by *Bacillus megaterium* M-3 (241.5 $), TV-91C (197.5 $) and TV-6D (192.7 $), while the lowest were obtained in control (123.4 $). Similar results were also determined for benefit/cost ratios. It was clearly seen that 4.2 $ income from each 1 $ cost was obtained from *Agrobacterium rubi* A-16 when benefit/cost ratio was taken into consideration. Plus, it can be clearly said that benefit/cost ratio was higher both in *Agrobacterium rubi* A-16 and other bacterial treatments than control. While the highest cost (2.0 $ per $unit^{-1}$) was obtained in control, the lowest cost for one plant (1.27 $ per $unit^{-1}$) was determined in *Agrobacterium rubi* A-16.

Consequently, the results show that all bacteria strains were found to be better to increase rooting percentage, in particular to increase root and shoot length of *Epipremnum aureum*. Inoculation with these bacteria strains, especially *Agrobacterium rubi* A-16, may also have a great potential for promoting root formation in *Epipremnum aureum* and according to the economic analyses, the highest net profit for potential producers was determined in the same bacteria. All the bacteria species tested in this work may also be used for promoting root formation in other ornamental species. Our study presents the first data on the effect of plant growth promoting rhizobacteria (PGPR) inoculation on rooting of *Epipremnum aureum* stem cuttings.

Table 2. Effect of bacteria inoculation on rooting percentage (%), root length (cm) and dry matter content of roots (%) of *Epipremnum aureum* cuttings.

Bacterial strains	Rooting (%)	Root length (cm)	Dry matter content of roots (%)
Control	36.67 ± 2.89 $_{d}$*	16.50 ± 0.20 $_{b}$	7.73 ± 0.15 $_{ab}$
Agrobacterium rubi (A-16)	75.00 ± 5.00 $_{a}$	23.90 ± 1.02 $_{a}$	8.33 ± 0.25 $_{a}$
Bacillus megaterium (TV-91C)	53.33 ± 2.89 $_{c}$	22.47 ± 1.45 $_{a}$	8.00 ± 0.40 $_{a}$
Bacillus megaterium (M-3)	61.67 ± 5.77 $_{b}$	21.83 ± 1.25 $_{a}$	7.27 ± 0.15 $_{b}$
Bacillus megaterium (TV-6D)	51.67 ± 2.89 $_{c}$	21.20 ± 0.20 $_{a}$	8.13 ± 0.35 $_{a}$

* Data were presented as mean ± standard error (SE). Means followed by different small letters in column are significantly different at $P < 0.05$.

Table 3. The effect of bacteria inoculation on new leaf number per cutting and leaf area (cm^2) of *Epipremnum aureum* cuttings.

Bacterial strains	New leaf number	Leaf area (cm^2)
Control	1.57 ± 0.06 $_{b}$*	8.41 ± 0.05 $_{b}$
Agrobacterium rubi (A-16)	2.30 ± 0.10 $_{a}$	8.99 ± 0.01 $_{b}$
Bacillus megaterium (TV-91C)	2.87 ± 0.47 $_{a}$	8.82 ± 0.16 $_{b}$
Bacillus megaterium (M-3)	2.73 ± 0.49 $_{a}$	10.32 ± 0.68 $_{a}$
Bacillus megaterium (TV-6D)	2.97 ± 0.37 $_{a}$	9.90 ± 0.58 $_{a}$

* Data were presented as mean ± standard error (SE). Means followed by different small letters in column are significantly different at $P < 0.05$.

Table 4. The effect of bacteria inoculation on shoot length (cm) and chlorophyll content (SPAD) of *Epipremnum aureum* cuttings.

Bacterial strains	Shoot length (cm)	Leaf Chlorophyll content (SPAD)
Control	2.93 ± 0.15 $_{d}$*	22.73 ± 0.92 $_{c}$
Agrobacterium rubi (A-16)	5.10 ± 0.20 $_{a}$	25.88 ± 0.56 $_{a}$
Bacillus megaterium (TV-91C)	3.60 ± 0.20 $_{c}$	25.97 ± 0.41 $_{a}$
Bacillus megaterium (M-3)	3.03 ± 0.06 $_{c}$	24.71 ± 0.20 $_{b}$
Bacillus megaterium (TV-6D)	4.33 ± 0.25 $_{b}$	22.60 ± 0.15 $_{c}$

* Data were presented as mean ± standard error (SE). Means followed by different small letters in column are significantly different at $P < 0.05$.

Table 5. Comparative cost and benefit analysis of plant growth promoting rhizobacteria use in *Epipremnum aureum* production.

Application	Control	A-16	TV-91C	M-3	TV-6D
1. 100 stem cuttings cost ($)	17.8	17.8	17.8	17.8	17.8
2. Perlite ($)	6.2	6.2	6.2	6.2	6.2
3. Peat ($)	8.0	16.2	11.5	13.4	11.2
4. Irrigation ($)	3.1	3.1	3.1	3.1	3.1
5. Bacterium ($)	0.0	3.7	3.7	3.7	3.7
6. Flowerpot ($)	5.7	11.6	8.2	9.6	8.0
7. Labor ($)	1.7	3.5	2.5	2.9	2.4
8. Variable cost (1+…+7)	42.5	62.0	52.8	56.6	52.4
9. Capital interest (8 × %6)	2.5	3.7	3.2	3.4	3.1
10.Total variable cost (8 + 9)	45.0	65.7	56.0	60.0	55.6
11. Greenhouse cost	27.8	27.8	27.8	27.8	27.8
12. General management costs (10 × 3%)	1.4	2.0	1.7	1.8	1.7
13. Total fixed cost (11 + 12)	29.1	29.7	29.5	29.6	29.4
14. Total cost (10 + 13)	74.1	95.5	85.5	89.5	85.0
15. Yield (Number of rooted cuttings)	37	75	53	62	52
16. Selling price ($ / plant)	5.3	5.3	5.3	5.3	5.3
17. Gross Revenue (15 × 16)	197.6	400.5	283.0	331.0	277.7
18. Net profit ($) (17 - 14)	123.4	305.0	197.5	241.5	192.7
19. Benefit / Cost ratio (17 / 14)	2.7	4.2	3.3	3.7	3.3

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