Growth Promoting of Some Ornamental Plants by Root Treatment with Specific Fluorescent Pseudomonads

M. Erhan Göre and Nedim Altin
Plant Protection Research Institute, 35040 Bornova, Izmir, Turkey

Abstract: The objective of the present study was to evaluate the ability of *Pseudomonas* to promote plant growth on *Pelargonium*, *Chrysanthemum* and *Dahlia* under commercial greenhouse conditions. The screening procedure consisted of two stages. In the first stage all the strains were tested in the two ornamental plants, *Pelargonium* and *Chrysanthemum*. In the second stage the most promising strain selected in the first stage was tested in *Dahlia*. Analysis of variance was done with the GLM (General Linear Models) procedure of SAS. Results of the first trial showed that *Chrysanthemum* plants treated with *Pseudomonas fluorescens* strain 51 increased plant height and number of flowers by 10% and 19%, respectively. Besides, increases as high as 13, 56 and 60% were obtained by *P. fluorescens* strain 148 applied by soil treatment, respectively for plant height, leaf surface area and number of flowers of *Pelargonium*. In the second trial, performed on *Dahlia*, plant raised from strain 51 treated pots recorded 27% increase in root length, 107% more flowers, 29 and 14% enhancement in fresh and dry weight, respectively. Moreover, the compatibility of the most efficient strain 51 with some fungicides, which are commonly sprayed on greenhouse was investigated under in vitro conditions. Fungicides did not adversely affect the colonial development of bacteria at concentrations as high as 100 μg mL⁻¹ in vitro.

Key words: Ornamental plants, PGPR, Pseudomonads

INTRODUCTION

Agriculture is increasingly dependent on the use of chemical fertilizers, growth regulators and pesticides to increase yield. This dependency is associated with problems such as environmental pollution, health hazards, interruption of natural ecological nutrient cycling and destruction of biological communities that otherwise support crop production. Hence, crop improvement and disease management have to be achieved in shorter intervals of time with fewer detrimental inputs. The use of bioresources to replace chemical pesticides, growth regulators and fertilizers is growing. In this context, Plant Growth Promoting Rhizobacteria (PGPR) are often novel and potential tools to provide substantial benefits to agriculture.

PGPR are naturally occurring soil bacteria that are able to colonize plant roots and improve plant growth when applied to roots, tubers or seeds (Enbak* et al.*, 1998; Kloepper* et al.*, 1980, Weller, 1998). In recent years, the concept of PGPR-mediated plant growth promotion is gaining worldwide importance and acceptance. The beneficial effects of PGPRs fall into two categories: direct plant growth promotion and plant disease suppression. The exact mechanisms of PGPR action are not yet fully understood, but they are reported to enhance plant growth by production of hormones, phosphate solubilization or nitrogen fixation. Disease suppression may be due to iron sequestration, production of antibiotics or induction of systemic resistance (Alstrom, 1991; Liu* et al.*, 1995; Reddy* et al.*, 1999; Vidyasekharan* et al.*, 2001; Zehnder* et al.*, 2000). The utilization of natural PGPR strains as inducers of plant defense responses may increase their applicability and offer a practical way to deliver growth promotion. Large numbers of bacteria, mainly *Pseudomonas fluorescens* and other sp.; *Bacillus* sp. and *Serratia marcescens* have been reported previously to act as PGPRs. In the present study, five plant growth-promoting rhizobacteria were evaluated under greenhouse conditions for their capacity to promote the growth of *Pelargonium peltatum* F1, *Chrysanthemum* (Virunga Yellow) and *Dahlia variabilis* Figaro plants.

MATERIALS AND METHODS

Bacterial strains and inoculum preparation: The PGPR strains chosen, *Pseudomonas putida* strain 17, *Pseudomonas fluorescens* strains 51, 116, 122 and 148, were obtained from Ege University, Faculty of

Corresponding Author: M. Erhan Göre, Plant Protection Research Institute, 35040 Bornova, Izmir, Turkey
Tel: 90 232 3880030 Fax: 90 232 3741653
Agriculture, Department of Plant Protection. All of the selected PGPR strains for evaluation have significantly promoted plant growth and reduced ascochyta blight of pea in the field conditions (Göre and Bora, 2004).

Fluorescent Pseudomonads were grown in Erlen-meyer flasks (250 mL) containing 100 mL of King’s medium B (KB) for 20 h on a rotary shaker at 24°C. At this stage, bacterial cells were active and were found in the exponential growth phase. The cells were pelleted by centrifugation at 10,000 g for 15 min at 4°C, suspended in sterile phosphate buffer (70 mM potassium phosphate buffer at pH = 7) and the concentration adjusted to 10^6 CFU mL^{-1} using a viable versus absorbance at 600 nm curve specific for P. fluorescens and P. putida strains. Appropriate dilutions from the original concentration were performed as described by Thompson (1996).

Experimental design and cultural practices: The experiments were conducted at the commercial greenhouse conditions, Inciralti, Izmir, Türkiye, during the years 2004 to evaluate 5 PGPR strains and a control. The experimental design consisted of 10 replications per treatment arranged in a randomized block design. Three week old plants were transplanted into larger 600 mL pots containing planting mix and drenched with 100 mL of a 10^6 CFU mL^{-1} bacterial suspension per plant. Bacterial inoculation was repeated one week later, in the same conditions. A water-buffer solution was applied to control plants. Plants were grown under 50% soil water holding capacity, 85-90% relative humidity, temperature of 18-23°C during the daytime and 14-16°C the nighttime.

The screening procedure consisted of two stages. In the first stage all strains were tested in the two ornamental plants, P. peltatum F1 and Chrysanthemum (Virunga Yellow). In the second stage, the most promising strain selected in the first stage was tested in D. variabilis Figaro. The first trials were ended 50 sec was ended 30 days after first inoculation. Observations were made all along the experiments, measuring days from inoculation to first flower, diameter of each plant’s leaf canopy, leaf surface area, plant height, root length, fresh and dry weight, number of leaves and flowers.

Mechanisms of PGPR: The phosphate solubilizing test was done using Pikovskaya’s medium (Pikovskaya, 1948). Agar plates were prepared and the bacterial strains were spot inoculated at the centre of the plates and incubated for 5-6 days. The plates were observed for a clearing zone around the colony and the diameter of the clearing zone was measured. Hydrogen cyanide production was assessed by the picrate method (Sneath, 1966). Tubes containing LB agar (Luria Bertani agar medium) and a filter paper strip impregnated with 0.5% picric acid and 2% NaCO\textsubscript{3}, introduced into the test tube screw cap, were inoculated with the bacteria. Upon incubation at 24°C for 3 days production of cyanide was detected as a change of the filter paper color from yellow to orange-brown. Indole Acetic Acid (IAA) and related compounds were identified upon colony growth in LB agar amended with 5 mM 1-tryptophan and overlaid with an 82 mm dia nitrocellulose membrane disk (Brito Alvarez et al., 1995). Agar plates were inoculated with bacterial cultures and incubated at 24°C for 3 days. Then, the membranes were removed and placed on Whatman filter paper impregnated with the Salkowskii reagent. Strains producing IAA or analogs were identified by a pink to red color in the filter surface. Siderophore production was determined using Chrome Azurol S (CAS) medium (Schwyn and Neilands, 1987). CAS agar plates were inoculated and incubated at 24°C for 5 days. Strains exhibiting an orange halo were positive for siderophore production.

In vitro fungitoxicity tests: The fungicides; mycelobutanil, captan and tebuconazole were kindly supplied by Hektas Crop Protection AG (Gebze, Turkey), diniconazole and thiophanate methyl by Sumitomo (Osaka, Japan), iprodione by Bayer Crop Science AG (Leverkusen, Germany) and benomyl by Dupont (Wilmington, USA). Stock solutions of these fungicides were made in ethanol, with exceptions of benomyl and iprodione which were dissolved in acetone. The sensitivity of strain 51 to the fungicides was assessed on KB plates containing a range of concentrations of each fungicide (0, 1, 3, 10, 30 and 100 µg mL^{-1}). The fungicides were added aseptically to sterilized growth medium from stock solutions, prior to inoculation. At least six concentrations with ten replicates for each fungicide were used in order to obtain the respective fungitoxicity curves. The experiments were conducted in 9 cm petri dishes, inoculated with 0.1 mL bacterial suspensions. The effect of the fungicide on growth was determined by measuring the number of colonies, after incubation for 3-4 days at 24°C in the dark.

Statistical analysis: The statistical analyses were performed with SAS software (SAS Institute, 2000 Inc, Cary, NC, USA). Data collected were subjected to analysis of variance using PROC, ANOVA and treatment means were separated using Duncan’s multiple range test at p = 0.05.

RESULTS

To examine the effect of the Pseudomonas strains on plant growth promoting activity, in the first trial, 5 PGPRs were inoculated on Chrysanthemum and Pelargonium plants at 3 weeks old. Table 1 showed that
Pelargonium plants exhibited significant increase in growth, mainly in the production of more leaf surface area and more flowering when *P. fluorescens* strain 148: 51 and 122 were applied to soil. 148: 51 and 122 augmented, respectively 56, 18 and 10% of leaf surface area as well as 60, 100 and 20% of number of flowers. Also, highest plant height was observed in 148 treated plants with an increase of 13% over the control. The lowest efficacy was recorded in strain 17 and 116. Treatment with these isolates enlarged the leaf surface area but decreased the number of flowers and plant height.

In *Chrysanthemum*, significant increase in plant growth parameters was registered in *P. fluorescens* strain 51 giving 10 and 19% increase in plant height and number of flowers, respectively (Table 2). In addition, it significantly decreased the No. of days required for flowering (50%), increased the number of basal branches (branches from the rosette) and decreased the number of aerial branches (branches above the rosette). Moreover, plants treated with 51 flowered on average 6 day faster than control plants. This was followed by 17 and 122 which resulted in significant reduction in number of days required for 50% flowering and augmented, respectively 5 and 7% of plant height (Table 2).

Based on the plant growth promoting activity on *Pelargonium* and *Chrysanthemum*, fluorescent pseudomonas strain 51 was selected for the second greenhouse trial which was performed on *Dahlia*. Also, *P. fluorescens* strain 51 treatment was found to be very effective in improving the plant growth characters of *Dahlia* compared to non-treated control. Plant raised from 51 treated pots recorded 27% increase in root length, 107% more flowers, 29 and 14% enhancement in fresh and dry weight, respectively (Table 3). It also decreased the number of days required for flowering (7 days).

**Characterization of the strains:** Of the 5 growth-promoting isolates, 5 produced siderophores, 2 produced auxins, 3 solubilized tricalcium phosphate and 4 produced HCN *in vitro* (Table 4).

### Fungicide sensitivity of the most efficient strain:

Among the PGPRs, significant increase in plant growth parameters was registered in *P. fluorescens* strain 51. To be integrated into most production systems, strain 51 must be compatible with other management practices. For this purpose, we assessed the compatibility of strain 51 with seven fungicides recommended for ornamental plants (Table 5). Fungicides tested did not kill strain 51 at the concentrations tested in the *in vitro* experiment. Visual observations on fluorescent pigmentation, growth and population counts of the bacteria revealed that benomyl, thiophanate methyl and tebuconazole gave the
Table 4: Secondary metabolite production of the fluorescent *Pseudomonas* in vitro

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain</th>
<th>Plant</th>
<th>Organ</th>
<th>HCN</th>
<th>IAA</th>
<th>Std</th>
<th>Phos.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. putida</em></td>
<td>17</td>
<td>Tobacco</td>
<td>Root</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><em>P. fluorescens</em></td>
<td>51</td>
<td>Pea</td>
<td>Root</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>116</td>
<td>Pea</td>
<td>Root</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>122</td>
<td>Pea</td>
<td>Root</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>148</td>
<td>Pea</td>
<td>Root</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

1Secondary metabolite production: Hydrogen Cyanide (HCN), Indole Acetic Acid (IAA), Siderophores (Std.) and Phosphate solubilization (Phos.)

Table 5: *In vitro* effects of some fungicides on colonial development of *P. fluorescens* strain 51

<table>
<thead>
<tr>
<th>Proprietary name</th>
<th>Active ingredient</th>
<th>(10 ppm)</th>
<th>(30 ppm)</th>
<th>(100 ppm)</th>
<th>(300 ppm)</th>
<th>(1000 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nimbus 24 EC</td>
<td>Mycelubanil</td>
<td>27.5</td>
<td>30.3</td>
<td>21.8</td>
<td>19.0</td>
<td>9.6</td>
</tr>
<tr>
<td>Spotted 5 EC</td>
<td>Diniconazole</td>
<td>22.8</td>
<td>29.0</td>
<td>18.8</td>
<td>24.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Revul 50 WP</td>
<td>Iprodione</td>
<td>35.0</td>
<td>31.4</td>
<td>28.4</td>
<td>29.6</td>
<td>16.2</td>
</tr>
<tr>
<td>Benlate 50 WP</td>
<td>Benomyl</td>
<td>24.0</td>
<td>27.8</td>
<td>22.4</td>
<td>39.2</td>
<td>28.0</td>
</tr>
<tr>
<td>Captain 70 WP</td>
<td>Captain</td>
<td>33.0</td>
<td>26.4</td>
<td>18.6</td>
<td>6.8</td>
<td>12.4</td>
</tr>
<tr>
<td>Miracle 75 WP</td>
<td>Telezinazole</td>
<td>25.2</td>
<td>32.0</td>
<td>25.8</td>
<td>26.6</td>
<td>32.6</td>
</tr>
<tr>
<td>Sunstop 70 WP</td>
<td>Thiosulfate methyl</td>
<td>32.2</td>
<td>25.25</td>
<td>20.3</td>
<td>30.7</td>
<td>35.0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>28.6</td>
</tr>
</tbody>
</table>

1All values are means of two experiments with 10 replications in each experiment

best compatibility. Iprodione was found to be partly compatible with the strain 51 and reduced final count only at 100 µg mL\(^{-1}\). However, diniconazole was found to be most toxic to strain 51 and significantly reduced growth rate and final colony count at 100 µg mL\(^{-1}\) compared to growth on unamended medium. Captain and mycelubanil reduced final colony count at 30 µg mL\(^{-1}\) or greater.

**DISCUSSION**

Present results clearly demonstrate that PGPRs can be used under greenhouse conditions to enhance growth of *Pelargonium, Chrysanthemum* and *Dahlia*. Growth stimulation effects reported in the literature by the use of PGPR strains are similar to those reported by us (Dedej et al., 2004; Scherm et al., 2004). However, most reports deal with industrial and vegetable plants, including chick-pea, soybean, sugarcane, pea and canola (Dileep Kumar and Dube, 1992; Dileep Kumar et al., 2001; Gore and Bora, 2004; Kloepper et al., 1989; Mirza et al., 2001).

The effect of *Pseudomonads* was dependent on the rootstock type. According to analysis of variance, the interaction between strain and rootstock was significant (p<0.001). Among the strains, 51 was effective on three plants rootstock, 148 was effective only in *Pelargonium* and others were partly effective in some plant systems. Similar results describing strain-host plant specificity have been reported in other plant systems such as several herbaceous crops (Howie and Eshandi, 1983; Kloepper, 1996). This specificity appears to be related to the different composition of rhizosphere exudates depending on the plant species which affect the levels of colonization and subsequently the efficacy of the PGPR strains (Latour et al., 1996), or to the specific compounds present in the exudates that may stimulate in the bacteria the synthesis of secondary metabolites implicated in the plant growth promotion (Van Overbeek and Van Elsas, 1995).

The best strains were very diverse in secondary metabolite production. This is in agreement with other studies that have found a wide functional and genetic diversity between PGPR strains (Ellis et al., 2000; Rainey et al., 1994). However, in some strains certain phenotypic characteristics could be associated to rootstock growth promotion. Production of IAA *in vitro* by strain 51 may be implicated in the promotion of rootstock growth. Other reports indicate that while many plant growth-promoting bacteria can produce at least small amounts of IAA, high IAA producers are often inhibitory to root growth (Barazani and Friedman, 1999). Therefore, the role of IAA is not always consistent with plant growth promotion among bacteria and may depend on the plant species because plant responses to hormones vary considerably (Gutiérrez-Manero et al., 1996). However, more specific research should be conducted to prove its direct implication. The production of cyanide by strain 17 and by most of the *P. fluorescens* strains greatly contrasts with its ability to promote growth. Cyanide production can increase the fitness of the PGPR strains and enhance the suppression of deleterious rhizobacteria (Mazzola et al., 1992) or can stimulate the exudation of nutrients from plants (Åström, 1991). However, cyanide production has been reported to cause deleterious effects on plant development by inhibition of root growth on some
plant-PGPR systems (Schappers et al., 1991). All strains produced siderophores. Siderophores can sequester iron in the rhizosphere making it unavailable to other rhizosphere microorganisms, then increasing the fitness of the PGPR strains (Kloepper et al., 1990). In addition, strains 51, 116 and 148 were efficient phosphate solubilizers. As reported by Richardson (2001) and Diby Paul et al. (2001), P solubilization by rhizosphere microorganisms may play significant roles in plant growth promotion.

It is a general belief that fungi or bacteria used as biocontrol or plant growth promoting agents should not be mixed together with any chemical pesticides and farmers are advised in the same way. Such a recommendation will limit the use of these agents in crops like ornamental plants where chemical pesticides are frequently applied as combined spray. Hence, it was felt necessary to study the compatibility of *P. fluorescens* strain 51 with commonly used fungicides. Strain 51 was found to be compatible with benomyl, tebuconazole, thiophanate methyl and partly with iprodiene. This is supported by the findings of Laha and Venkataraman (2001) that carbendazim was compatible with *P. fluorescens*. Significantly higher bacterial population was obtained in 100 μg mL⁻¹ carbendazim amended KB than in 50 μg mL⁻¹ amended KB or KM alone. In the Mathew (2003) studies, *P. fluorescens* was highly found compatible with 100 μg mL⁻¹ carbendazim, mancozeb and imidacloprid, and maximum bacterial count was recorded with these treatments.

The screening procedure used in this study was performed in a pathogen-free environment in a commercial nursery and no artificial inoculation of root pathogens was done. Thus, the theoretical benefits of certain antagonistic properties or antimetabolite production, may not be expressed in the environmental conditions used. It is expected that growth-promoting strains with high antimicrobial potential will provide additional advantages to *Chrysanthemum*, *Pelargonium* and *Dahlia* plants in the presence of root pathogens or deleterious bacteria. However, further studies are required to show if the suppression of specific pathogens of *Chrysanthemum*, *Pelargonium* and *Dahlia* rootstock from the root system increase the effect of growth promotion. To investigate the involvement of bacterial metabolites in rootstock growth promotion, experiments using reductionist molecular techniques will be performed.

Finally, the fact that *P. fluorescens* strain 51 was effective and consistently enhanced development of *Chrysanthemum*, *Pelargonium* and *Dahlia* rootstock after irrigation with relatively diluted bacterial suspension opens the possibility of its use in commercial ornamental nurseries. The effect of this strain on plant rootstock development is particularly interesting because an optimal growth during the first month is essential for good establishment in the greenhouse and also it is an advantage to shorten the time required for plant production. However, additional work has to be done to set-up suitable methods of mass production and formulation of the bacteria to be used in practice.

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REFERENCES


