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Rhizobacteria promoted yield of cucumber plants grown in perlite under Fusarium wilt stress

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ABSTRACT

This study was carried out to determine the effects of plant-growth-promoting rhizobacteria (PGPR) on cucumber (*Cucumis sativus* L. cv. Sardes F_1) production in perlite under unheated greenhouse conditions. Four native bacterial strains (18/1K: *Pseudomonas putida*, 62: *Serratia marcescens*, 66/3: *Bacillus* spp., 70: *Pseudomonas fluorescens*) and one commercial product (FZB24: *Bacillus amyloliquefaciens*) were tested. Rhizobacteria inoculation took place before sowing and after transplanting. Plants were affected by *Fusarium oxysporum* f.sp. *cucumerinum* occurred naturally and effects of PGPR on cucumber yield were found statistically significant. Plants inoculated with *Pseudomonas putida* strain 18/1K and *Serratia marcescens* strain 62 gave significantly higher yield compared to the control plants.

1. Introduction

Soilless culture has been increasingly popular in protected culture among commercial growers since it eliminates the problems originated from soil. Although it provides disease-free start to cultivation, but root diseases still can be major problem due to insufficient sterilization and/or contamination of the growing system. The strategy is to keep the growing systems as clean as possible; however, the sterility concept commonly used in soilless system has been broken with increased knowledge on the beneficial microflora. The new trend is introduction or stimulation of microflora in soilless system to induce resistance of plants against biotic and abiotic stress factors, and increase plant growth and yield (Alsanius et al., 2004; Koohakan et al., 2004; Deniel et al., 2006; Gravel et al., 2006; Sopher and Sutton, 2011).

Among beneficial microorganisms for a sustainable agriculture, plant growth-promoting rhizobacteria (PGPR) are of great importance (Lucy et al., 2004). These bacteria colonize roots and cause either plant growth promotion or biological control of plant disease (Lee et al., 2010). Many PGPR have both of these effects on plants. Plant growth promotion is based on increasing nutrient cycling and/or producing biologically active substances such as auxins and other plant hormones (Zhang et al., 2004; Khalid et al., 2004). PGPR mediated biological control is caused by several mechanisms such as competition, producing antibiotic substances and siderophores, and induced resistance (Pieterse et al., 1996; Zhang et al., 2004; Bakker et al., 2007).

It is reported that studies on using microorganisms have been generally realized in growth chambers, laboratories or small field plots; and it is not possible in the farm scale to reach the same success obtained under controlled conditions (Vannacci and Gullino, 2000). More studies for specific crops in various geographical areas are needed to increase adoption of microbial inoculants (Kloepper et al., 2004). This study was carried out to determine the effects of PGPR on cucumber production in perlite under unheated greenhouse conditions.

2. Materials and methods

2.1. Bacterial strains

Four bacterial strains from the collection of the Department of Plant Protection, Agricultural Faculty of Ege University (18/1K: *Pseudomonas putida*, 62: *Serratia marcescens*, 66/3: *Bacillus* spp., 70: *Pseudomonas fluorescens*) and one commercial product (FZB24: *Bacillus amyloliquefaciens*) were tested in this study. Bacterial strains were selected according to the results of our previous study on their effect on seedling growth (Kidoglu et al., 2008).

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2.2. Plant material and growing conditions

The research was carried out in a polyethylene covered, non-heated greenhouse located at the Department of Horticulture, Ege University (38°27′16.2″N, 27°13′17.8″E). Cucumber (*Cucumis sativus* L. cv. Sardes F_1) was used as plant material. Seedlings were produced in a commercial nursery, transferred to the experimental greenhouse at the five true-leaf stage and transplanted in plastic pots filled with perlite (8 L/plant). Pots were arranged in order to provide plant numbers of 3.48 per m^2 equivalent to 34,800 plants per hectare. Experiment including 6 treatments (5 PGPR strains and control) was set up according to randomized blocks with four replicates and each plot had 9 plants.

Complete nutrient solution was used to cover water and nutrient requirements of the plants (Papadopoulos, 1994) and applied *via* drip irrigation system with 2 L/h flow rate. The amount of nutrient solution was adjusted according to the drainage volume kept around 20%, and surplus solution was allowed to run to waste (open system).

2.3. Bacterial inoculation

Bacterial inoculation was carried out before sowing and after transplanting (Cummings et al., 2009; Singh et al., 2003). PGPR strains were grown on King's medium B for 24 h at 24 °C. Bacterial inoculants were suspended with 5 mL carboxyl methyl cellulose (CMC, 1.5%). The concentration of bacterial cells in the suspension was adjusted by diluting with sterile deionised water, thus a final concentration of 10^9 CFU/mL was obtained (Callan et al., 1990).

Seeds were sterilized in 1% hypochlorite acid for 2 min and rinsed in distilled water. Seeds were then treated with the bacterial suspensions at the concentrations of 10^9 CFU/mL in Erlenmeyer flasks by shaking for 30 min at 140 rpm. In the control treatment (no-PGPR), seeds were shaken with CMC. After shaking, seeds were left on blotting papers for 24 h under sterile cabinet before sowing. Seedling production were realised in a commercial nursery. Seeds were sown manually in a commercial medium (a mixture of peat and perlite) in plastic viols. After germination, seedling trays were transferred to greenhouse used for organic seedling production and no pesticides were applied.

The second bacterial inoculations were made 1 week after planting as root drenches. Each treatment was applied with 50 mL/plant of bacterial suspensions at the concentrations of 10^9 CFU/mL. Water was applied in the same way as a non-treated control.

2.4. Variables measured

- Root colonization and population dynamics of native PGPR strains on cucumber roots: root samples were taken monthly in order to study colonization by introduced rhizobacteria during the growing period. Root samples were collected and placed into sterile flasks, 100 mL of 0.1 M phosphate buffer (pH 7.1) was added to each flask. Flasks were placed on a rotary shaker at 150 rpm for 10 min. Samples were diluted, spread on two replicate Petri dishes containing Kings medium B supplemented with rifampicin (100 μg/mL) and bacterial colonies, which were rifampicin resistant mutants of tested PGPR strains were enumerated (Stockwell et al., 1998).
- Production of IAA by PGPR: bacterial isolates from the collection of Ege University were assayed for their ability to produce indole-3-acetic acid (IAA) *in vitro* which was determined quantitatively according to Bric et al. (1991) and Asghar et al. (2002), and expressed as mg/L. Bacterial isolates were grown in liquid nutrient broth medium supplemented with L-tryptophan for 24 h. Then, the culture filtrates of the bacteria were obtained and measured by spectrophotometer (535 nm).
- Yield: plants were grown for 14 weeks and yield was recorded as fruit weight and number of fruits per plant.
- *In vitro* antagonistic activity of the PGPR strains against *Fusarium oxysporum* f.sp. *cucumerinum* (FOC): plants were affected by FOC which occurred naturally. Therefore, *in vitro* experiments were setup to assay the effects of PGPR. We tested the inhibition of hyphae development of FOC, isolated from infected plants, by dual plate assay. *In vitro* antifungal activity was evaluated by measuring the diameter of fungal colony (mm) after the plates were incubated at 24 °C for 7 days. Antagonistic activity of the PGPR strains was estimated by the inhibition of the fungal growth in comparison to a solely cultivated fungal agar disk.

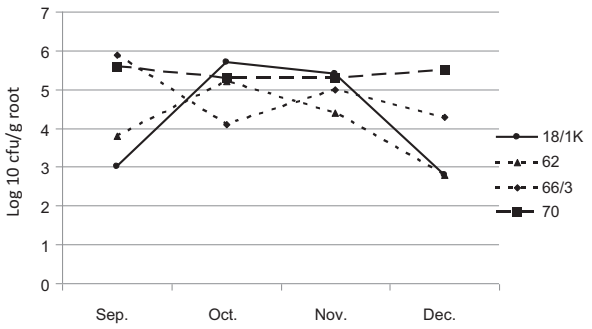

Fig. 1. Colonization of native rhizobacteria strains on cucumber roots and time-dependent population dynamics.

2.5. Statistical analysis

The yield data were subjected to ANOVA. Means were compared using Fisher's protected least significant difference (LSD). Significance was set at $P \leq 0.05$. Regression analyses were performed between *in vitro* production of IAA by native PGPR strains and yield.

3. Results

3.1. Population dynamics of native PGPR strains on cucumber roots and their ability to produce IAA

Colonization dynamics of PGPR strains from Ege University on cucumber roots are shown in Fig. 1. Populations of PGPR on roots of cucumber seedlings at planting changed between 10^3 and 10^6 CFU/g root. It was determined that PGPR strains tested in this study could survive on cucumber plants through the vegetation period lasting 3 months. The best colonization level was obtained by 70.

It was determined that all PGPR strains from Ege University had the ability to produce IAA. The highest IAA production were realized by 62 (0.700 mg/L) followed by *P. putida* strain 18/1K (0.610 mg/L) and 70 (0.160 mg/L), while 66/3 produced the lowest amount (0.065 mg/L).

3.2. Yield

There were significant differences between treatments through the harvesting season. Yield changes were presented as 2 weekly cumulative yields (Table 1). Plants inoculated with 18/1K, 62, 70 and FZB24 gave higher yield compared to the control plants by the first 2 weeks. Increases varied from 78.5% (70) to 121.1% (18/1K) in native PGPR strains and commercial product of FZB24 was associated with a 66.1% higher yield compared to the control treatment. 18/1K and 62 ranked at the top also in further weeks. Yield increases