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POTENTIAL OF *PSEUDOMONAS* AND *BACILLUS* ISOLATES AS BIOCONTROL AGENTS AGAINST FUSARIUM WILT OF EGGPLANT

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) are beneficial bacteria found on plant roots that induce growth by a wide variety of mechanisms. Ten isolates of Pseudomonas and Bacillus were isolated from eggplant rhizosphere soil and evaluated for their volatile compound efficacy against mycelial growth of Fusarium oxysporum f. sp. melongenae (Fomg) and tested for their ability to colonize eggplant (Solanum melongena L.) roots in vitro. PGPR strains inhibited mycelial growth of the target pathogen and the percentage of inhibition varied from 38 % to 72 % by means of inhibitory activities. Pseudomanas aeruginosa (P07-1) and P. putida (P11-4) were determined to be successful colonizers in eggplant seedlings. Also, a pot experiment was conducted to assess the induced resistance to Fusarium wilt of eggplant by PGPR strains. Among the PGPR isolates, P. aeruginosa (P07-1), P. putida (P11-4), P. aeruginosa (85A-2), Bacillus amyloliquefaciens (76A-1) and B. cereus (B10a) significantly reduced Fusarium wilt disease incidence by up to 85 %. However, their combination was not more effective in suppressing the disease than the application of those isolates alone. The induction of peroxidase (POX, EC 1.11.1.7) and polyphenol oxidase (PPO, EC 1.14.18.1) enzymes was found to be significantly higher in comparison with the control group. Similarly, the proline levels in eggplants showed an increasing trend with the above isolates. However, catalase (CAT, 1.11.1.6) activity was not found to significantly contribute to the induced resistance mechanism. This study revealed that the promising PGPR isolates could be potentially very useful for the biocontrol of Fomg via enhancing disease resistance in eggplant plants.

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Keywords: PGPR, *Pseudomonas* spp., *Bacillus* spp., Fusarium wilt, defense enzymes, eggplant

Introduction

Fusarium oxysporum f. sp. *melongenae* (*Fomg*) is the most destructive disease agent of Fusarium wilt of eggplant (*Solanum melongena* L.). The soil-borne fungus invades the vascular bundles, causes severe wilting and death of the above ground parts of plants by blocking the xylem transport system (1). It is extremely difficult to control soil-borne fungi via conventional strategies such as the use of synthetic fungicides, etc. Since their spores are able to survive for many years in the soil, biological control strategies for this pathogen should, therefore, be carefully selected and handled in an eco-friendly way instead of using chemical fungicides. The application of microorganisms as biocontrol agents is important, since they may increase beneficial microbial activity which extends for a long period of time. A group of bio-fertilizers, in dried powder formulations, also known as plant growth-promoting rhizobacteria (PGPR), have been applied to seeds and soil successfully for a number of years (20).

Root-colonizing plant-beneficial bacteria and fungi may provide good protection for plants against root-attacking pathogens (10). In the last decade, many strains of plant growth-promoting rhizobacteria were reported to induce systemic resistance against a broad spectrum of soil-borne and foliar pathogens (25). The plant and bacterial interactions in the rhizophere are important for plant health and resistance to disease (20). For example, non-pathogenic PGPRs are known to rapidly colonize the rhizosphere and enhance plant resistance, which is termed induced systemic resistance (ISR) (20), while pathogen-induced resistance is called systemic acquired resistance (SAR) (16). Both SAR and ISR are expressed against a broad spectrum of root and foliar pathogens (34). They are both related with the induction of pathogenesis-related (PR) proteins. Moradi et al. (30) showed an increase in the induction of resistance to Fusarium wilt in chickpea by *B. subtilis*. They also demonstrated that this resistance was related to PR-proteins.

Various mechanisms are involved in the biological control of fungal pathogens by PGPR. These mechanisms include the production of secondary metabolites such as antibiotics, siderophores, hydrolytic enzymes, phytohormones, volatile extracellular metabolites, hydrogen cyanide and competition for nutrients, promotion of plant growth and, finally, induced resistance within the plants (10, 27). PGPR also induce ISR by triggering jasmonic acid (JA) and ethylene synthesis (34). Among rhizobacteria, fluorescent *Pseudomonads* and some species of *Bacillus* are considered to be a promising group for biocontrol studies (27). PGPR may also promote plant growth by providing nitrogen (13), simplifying nutrient uptake (5),

helping the solubilization of phosphorus (36, 37) as well as by being involved in the production of phytohormones (33).

Although *Fomg* is one of the major causes of eggplant wilt disease in Turkey, so far few studies have been carried out regarding the biological control via application of PGPR. In our previous study, we identified some PGPR strains showing high antifungal activities against *Fomg* in *in vitro* (44). In the present study, we evaluated ten PGPR strains alone or in combination to control Fusarium wilt of eggplant in pot experiments and assessed oxidative stress and defense-related responses such as catalase (CAT, E.C. 1.11.1.6), peroxidase (POX, E.C. 1.11.1.7) and polyphenol oxidase (PPO, E.C. 1.14.18.1) activity, as well as various other biochemical metabolites such as proline, to gain a better insight into the control mechanism.

Materials and Methods

Plant material and growth conditions

Eggplant (*Solanum melongena* L. cv. Kemer *susceptible to Fomg*) seeds were surface sterilized with 1 % sodium hypochlorite (v/v) for 30 min and sown in a soil mixture containing sand, perlite, and peat compost (1:1:2) and kept in a growth chamber (mean temperature: 25 ºC, relative humidity: 70 % to 90 %, 12 h photoperiod, 50 Klux·m^{-2} to 60 Klux·m^{-2}). Four-week-old plants at the 5–6 leaf stage were then transplanted to pots (8.5 cm in diameter) containing a mixture of the above ingredients.

Bacterial and fungal isolates

Rhizobacteria were previously isolated from eggplant rhizosphere soil on King's B medium (KBM) (Merck, Darmstadt, Germany) (44). Rhizobacterial identification was carried out using a Biolog GEN III microbial identification system (Biolog GEN III technology, USA) and the reading of ID results was conducted using the MicroStation semi-automated identification system, which was used for identification of the bacterium based on its phenotypic pattern. Basically, each bacterium strain from the nutrient agar (NA) slant was cultured on the plates and incubated for 24 h at 35 °C. After the incubation period, colonies of bacteria were swabbed from the agar surface and suspended into IF-A (inoculating fluid, FocusBIOTECH) at cell densities of 98 %. Following this, 100 µL of the bacterial suspension were pipetted into each reservoir of the GEN III microplates, using a digital multichannel pipette, and then incubated into the OmniLog system for 30 h to 36 h at 35 °C. The Biolog GEN III microplate system analyzes a microorganism in 94 phenotypic tests: 71 carbon source utilization assays and 23 chemical sensitivity assays. The test panel provides a phenotypic fingerprint of the microorganism that can be used to identify it at the species level (www.biolog.com).

Ten PGPR strains were selected and used in this study: two strains of *P. putida* (P11-4, P13-1), four strains of *P. aeruginosa* (56K-3, 85A-2, P07-1, P07-4), *B. amyloliquefaciens* (76A-1), *B. cereus* (B10a), *B. subtilis* (B379-c), and *Pseudomonas* sp. (P48-2). The combined effects of *Pseudomonas* and *Bacillus* spp. were also evaluated. PGPR strains were stored in KBM (19) amended with 20 % glycerol at -80 ºC. All PGPR strains were maintained in KBM for 48 h. Bacterial cells were then collected by centrifugation at 8 000 *g* for 10 min and suspended in $MgSO_4$ (10 mmol·L^{-1}) and adjusted to 10^8 cfu·mL^{-1} (40).

Fomg was proved to be a highly virulent pathogen, according to our previous report (2). The inoculum of pathogen was prepared by culturing the fungus on PDA medium for 7 days. Specimens were then incubated at 24 °C, and the pathogen conidial suspension was prepared by pouring 10 mL of sterile distilled water into each Petri dish. The conidial suspension was adjusted with sterile distilled water to a 10^6 mL^{-1} concentration of conidia and then the eggplant seedlings were inoculated by the root-dip method.

Bacterial antagonistic assay

The volatile compound produced by PGPR strains was determined by the inverted plate technique of Dennis and Webster (11). PDA plates were inoculated with *Fomg* (5 mm disc) obtained from 48 h cultures of PGPR grown in amended KBM. Control plates were inoculated with a 5 mm diameter disc of sterile PDA medium. The plates were sealed with a sterile band and the paired plates were incubated at 28 °C ± 2 ºC for 7 days with 10 replicates. After the incubation period, the radial growth of pathogen was measured and the inhibition percentage was calculated.

In vitro root colonization

PGPR strains were tested for their ability to colonize eggplant (*Solanum melongena* L.) roots *in vitro*, according to the methods developed by Montealegre et al. (29) with slight modifications. Eggplant seeds were surface sterilized with 70 % ethanol for 5 min and were subsequently sterilized with 1 % sodium hypochlorite for 1 min followed by rinsing twice in sterile distilled water for 2 min each. For each treatment, 30 seeds were transferred to a moist chamber after being placed on filter paper in 90 mm diameter Petri dishes and moistened with sterile distilled water. For inoculation, the PGPR inoculum was prepared as described above. A 1 mL aliquot of each inoculum was added to the seeds in the moist chamber and the plates were incubated at room temperature for 1 h to allow binding of the bacteria to the seed coat. The control seeds were similarly inoculated with sterile distilled water. PGPR-treated seeds and the control were incubated at 30 ºC for 5 days in the dark for root development. One centimeter of root from each treatment was aseptically excised, then transferred to an $MgSO_4$ (0.1 mol·L^{-1}) solution, and diluted serially. From each dilution, a 0.1 mL aliquot was plated on KBM media and the plates were incubated at 28 ºC for colony counts. The number of bacteria per milliliter of root segment (cm) was calculated as cfu·cm^{-1} root.

Disease suppression

The strains were evaluated for their induced response against *Fomg* after soil bacterizations. Eggplant seedlings were

inoculated using the liquid inoculum method. For this, 50 mL of PGPR suspension was drenched into the rhizosphere region of the eggplant 10 days after planting. A week later, 100 mL of *Fomg* suspension containing 10^6 cfu·mL^{-1} was inoculated with the standard root-dip inoculation method (2). Control plants were treated with sterile distilled water instead of PGPR suspension. Inoculated plants were grown in the growth chamber conditions described above. The experiment was conducted in a completely randomized plot design with three replications and was repeated twice. The symptoms of the disease were recorded at 3-day intervals for 3 weeks after the exhibition of the first symptoms in infected plants with a *Fusarium* yellow rating of 0 to 4, in which 0 = no lesions, 1 = slight leaf chlorosis and necrosis, 2 = vein clearing on outer leaflets, 3 = yellowing and dropping of leaves, 4 = dead plant.

Disease assessment and data analysis

Plants were evaluated individually and a mean percent disease severity was calculated for each assessment day based on the scale values according to the Townsend–Heuberger formula (42). The data were subjected to analysis with Levene's homogeneity of variance test then grouped by Duncan's multiple range test ($P \leq 0.05$) part of the SPSS software (SPSS Inc., Chicago, IL, USA) (14).

Assay of defense-related proteins and enzymes

Leaves were carefully removed at different time intervals (7, 14 and 21 days) after pathogen inoculation. A 1 g sample of leaves was weighed and ground with an ice-cold pestle and mortar with 10 mL of 50 mmol·L^{-1} phosphate buffer (pH 7.0). The homogenates were then centrifuged at 12 000 *g* for 20 min at 4 ºC. The supernatant was filtered through two layers of cheese-cloth and was used for determination of enzymatic activities as well as protein and proline content. The homogenized leaf tissues were stored in a deep freezer (-80 ºC) until use for further biochemical analyses.

Proline measurement

The proline content of the leaves was measured according to Bates et al. (4). Proline was extracted from 0.5 g of leaf sample by grinding in 10 mL of 3 % sulfosalicylic acid and the mixture was then centrifuged at 10 000 *g* for 10 min. Two milliliters of the supernatant were then added to test tubes in which 2 mL of freshly prepared acid-ninhydrin solution and 2 mL of glacial acetic acid were mixed. The tubes were placed in a water bath for 1 h at 90 ºC and the reaction was terminated in an ice-bath. The mixture was then extracted with 5 mL toluene and vortexed for 15 sec. After allowing the mixture to stand for at least 20 min in darkness at room temperature to separate the toluene and aqueous phase, the toluene phase was then carefully collected into the test tubes and the absorbance of the fraction was read at 520 nm with a spectrophotometer (Shimadzu UV-1700). The proline concentration in the sample was expressed on a fresh-weight (FW) basis. The standard curve was prepared by employing L-proline.

Measurement of CAT activity

Catalase (CAT, E.C. 1.11.1.6) activity was assayed according to Kato and Shimizu (18), by monitoring the consumption of H_2O_2 by measuring the decrease in absorbance at 240 nm with a UV spectrophotometer. The reaction mixture consisted of 0.2 mL of enzyme extract, 1.5 mL of phosphate buffer (pH 7.4, 0.1 mol·L^{-1}), and 1 mL of distilled water. The biochemical reaction was then started by adding 0.3 mL of 0.1 mol·L^{-1} H_2O_2 in the reaction system. Samples without H_2O_2 were used as blanks. The activity of CAT was calculated based on the differences obtained in OD_{240} values at 30 s intervals for 2 min after the initiation of the biochemical reaction, and the results were expressed as U mg^{-1} protein·min^{-1}. The extinction coefficient of 40 mmol·L^{-1}·cm-1 at 240 nm for H_2O_2 was used.

Assay of PPO

Polyphenol oxidase (PPO, E.C. 1.14.18.1) activity was assayed with 4-methylcatechol as a substrate, according to the method described by Zauberman et al. (45). The reaction mixture contained 0.5 mL of diluted enzyme extract, 2 mL of phosphate buffer (pH 7.0, 0.1 mol·L^{-1}) and 0.5 mL of 100 mmol·L^{-1} 4-methylcatechol. The increase in absorbance at 410 nm at 25 °C was recorded every 30 s for 2 min. Enzyme activity was expressed as changes in absorbance in mg^{-1} protein·min^{-1}.

Assay of POX

Peroxidase (E.C. 1.11.1.7) activity was measured according to the method of Cvikrová et al. (9). Two grams of leaf material was homogenized in 50 mmol·L^{-1} Na–K buffer (pH 7.0) and centrifuged at 12 000 *g* for 10 min at 4 ºC. From the resulting supernatant, 100 µL of leaf extract was mixed with the reaction mixture (13 mmol·L^{-1} guaiacol, 5 mmol·L^{-1} H_2O_2 and 50 mmol·L^{-1} Na-phosphate, pH 6.8). The absorbance was then read at 470 nm at 25 ºC for 1 min. The activity was expressed as ΔA_{470}·mg^{-1} protein·min^{-1}.

Protein determination

The protein content of the samples was determined according to the Coomassie Brilliant Blue G250 method, using bovine serum albumin as a standard. The absorbance was measured at 595 nm (7).

Results and Discussion

Effect of antagonistic compounds and root colonization

The mechanism of antibiosis by PGPR and the inhibition of many pathogens are generally mediated through toxic volatile substances and other metabolic ways such as competition for iron and nutrients, lysis, production of enzymes such as chitinase, glucanase, protease etc. (43). The antagonistic effect of compounds produced by the PGPR strains was evaluated using the paired plate technique. The results revealed that the PGPR strains inhibited the growth of *Fomg* to a varying extent, 38.75 % to 71.99 %. The higher inhibition effect of the antagonistic metabolites was demonstrated by the strains *P. aeruginosa* (P07-1 and 85A-2) and *P. putida* (P11-4), which

TABLE 1

Effect of antagonistic metabolites released from the PGPR strains on mycelial growth of *Fomg*

PGPRs	Isolate	RG (mm)	MI (%)	*In vitro* RC (log cfu/cm)
Pseudomonas aeruginosa	007-4	41.23^{de}	54.19	11.01^{a*}
Pseudomonas aeruginosa	007-1	25.21^{f}	71.99	09.85^{c}
Pseudomonas aeruginosa	56K-3	43.41^{d}	51.76	08.29^{d}
Pseudomonas aeruginosa	85A-2	26.17f	70.92	07.92^{e}
Pseudomonas putida	11-4	26.20^{f}	70.89	10.61^{b}
Pseudomonas putida	13-1	55.12^{b}	38.75	06.24^{h}
Pseudomonas sp.	048-2	50.47^{c}	43.92	07.82^{ef}
B. subtilis subsp. *subtilis*	379c	42.32^{de}	52.97	06.37^{gh}
Bacillus amyloliquefaciens	76A-1	40.12^{e}	55.42	06.42^{g}
Bacillus cereus	10a	42.69^{de}	52.57	07.72^{f}
Control	-	90.00^{a}	-	-

*Means within columns followed by the same letter are not significantly different according to Duncan's multiple range test ($P > 0.05$). Values represent means of ten replicates. RG: radial growth; MI: mycelial inhibition; RC: root colonization.

TABLE 2

Analysis of variance on the disease severity on pre-inoculated eggplant (cv. Kemer) with PGPRs against *Fomg*

PGPRs	Isolate number	DSI	Disease severity (%)	Effect (%)
Pseudomonas aeruginosa	P07-4	0.80^{bcd*}	20.45	78.14
Pseudomonas aeruginosa	P07-1	0.33^{h}	8.25	90.98
Pseudomonas aeruginosa	56K-3	0.73^{bcde}	18.25	80.05
Pseudomonas aeruginosa	85A-2	0.45^{fgh}	11.25	87.72
Pseudomonas putida	P11-4	0.40^{gh}	10.04	89.07
Pseudomonas putida	P13-1	0.86^{bc}	21.51	76.54
Pseudomonas sp.	P48-2	0.60^{defg}	15.78	83.61
B. subtilis subsp. *subtilis*	B379c	0.66^{cdef}	16.57	81.97
Bacillus amyloliquefaciens	76A-1	0.46f^{gh}	11.55	87.43
Bacillus cereus	B10a	0.53^{efgh}	14.12	85.52
Pseudomonas–Bacillus sp.	cmb	0.93^{b}	23.25	74.59
Control	-	3.66^{a}	91.53	-

*Means followed by different letters in the column are significantly different at $P \leq 0.05$ as determined by Duncan's multiple range test. DSI: disease severity index.

inhibited the pathogen *Fomg* by approximately 70 %. The strains of both *P. aeruginosa* (P07-4) and (56K-3) and *P. putida* (P13-1) demonstrated moderate inhibitory activity, which inhibited the mycelial growth of *Fomg* by approximately 50 %. *Bacillus* isolates (B10a, B379c, 76A-1) also suppressed the mycelial growth of the pathogen by over 50 %. The lowest activity of inhibitory compounds was observed in *P. putida* (P13-1) (**Table 1**). The statistical differences between the means of the PGPR strains were found to be significant (F (10,20) = 438.34, $P \leq 0.001$). Although the mechanism of PGPRs varies among genera, a common mechanism for suppressing the growth of pathogenic fungi is that they can produce enzymes which lyse fungal cells. For example, *Pseudomonas stutzeri* produces extracellular chitinase and laminarinase which lyse the mycelia of *Fusarium solani* (26).

In addition to the effect of growth promotion exerted by PGPRs, which is related to the release of growth factors, they are also able to metabolize numerous and varying carbon sources, which shows great competence in colonizing the rhizosphere. The *in vitro* root colonization study demonstrated that PGPR isolates were quite effective as root colonizers. Only five days after germination, bacteria cells were counted in the eggplant roots. *P. aeruginosa* (P07-1) (10.61 cfu·cm^{-1} roots) and *P. putida* (P11-4) (11.01 cfu·cm^{-1} roots) were

determined to be successful colonizers in eggplant seedlings (**Table 1**). Similarly, the *B. subtilis* isolate GBO3 was found to suppress *F. oxysporum* f. sp. *ciceri* (17) and the same isolate also activated the ISR mechanism in *Arabidopsis* by means of some volatile metabolites (8). Someya et al. (38), on the other hand, demonstrated that certain *Bacillus* spp. can degrade chitin, the major cell wall component of phytopathogenic fungi. Earlier, it was reported that lysis of fungal mycelium was associated with chitinolytic enzyme production of *Bacillus* strains (3). Exactly how a beneficial bacterium induces changes in plant root morphology and colonization is not yet clearly understood. Bacterial population of hormone-like substances and their ability to stimulate endogenous hormone levels were believed to play an important role in this process (12). However, recently nitric oxide, diffusible gas, has been shown to act as a signaling molecule in an IAA-induced pathway involved in adventitious root development (28). It has been reported that *Pseudomonas* spp. in general has an antagonistic effect on several fungi such as *Fusarium* spp, *Pythium ultimum*, *Alternaria* spp., and *Botrytis cinerea*, suggesting that the diffusion of antifungal compounds into the neighboring medium plays an important role (15). It has been reported that the efficient root colonization largely depends on the diffusion rate and speed of antibiotics produced by PGPR around the root (21). Lugtenberg et al. (23) suggested that the fast growing rhizobacteria might compete with fungal pathogens by competition for carbon and energy sources and deliver antifungal metabolites to the root system which facilitate biocontrol.

Disease assessment

The analysis of variance on the disease severity of pre-inoculated eggplants with PGPRs against *Fomg* is given in **Table 2**. Data indicated that there were significant differences ($P \leq 0.05$) among the PGPR strains, although all PGPR strains enhanced the resistance of eggplant to *Fomg* [(F (11,22) = 143.71, $P \leq 0.001$)]. Among the PGPR isolates, *P. aeruginosa* (P07-1), *P. putida* (P11-4), *P. aeruginosa* (85A-2), *B. amyloliquefaciens* (76A-1), and *B. cereus* (B10a), significantly reduced the disease incidence by up to 85 % in pot experiments. The results showed that the combination of these two species against *Fomg* did not result in any significant differences in respect to disease resistance as compared to the efficacy of either of those isolates alone (**Table 2**). Similar observations were also made by Maina et al. (24), who reported that *P. putida* successfully reduced *Fusarium* wilt disease in induced tomato seedlings.

In other similar studies, *Bacillus* sp. was used as a biocontrol organism and successfully reduced Fusarium wilt of banana (32) and chickpea (30) *in vitro*. The mixture of *Bacillus* sp., *Pseudomonas* sp. and *Gliocladium* sp. reduced the severity of *F. oxysporum* f. sp. *cubense*. However, their combination was not more effective in suppressing the disease than the application of *Pseudomonas* alone (32).

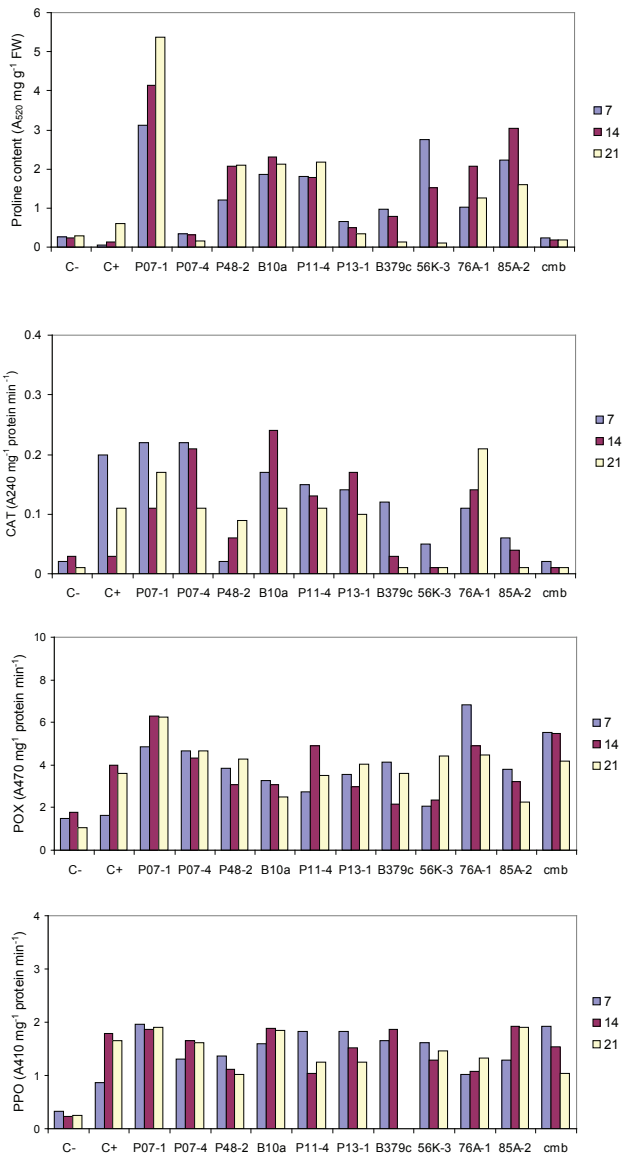


Fig. 1. Effect of PGPR treatments on praline content and CAT, POX and PPO enzyme activities in eggplants 7, 14 and 21 days following inoculation with *Fomg*.

Induction of defense enzymes in eggplant against *Fomg*

The levels of protein, proline, CAT, POX and PPO were analyzed in leaf tissues at different times after the challenger inoculation. The assay of defense enzymes revealed that PGPR strains induced a greater amount of enzymes in PGPR-treated eggplants than that observed in the control plants. The induction of POX and PPO enzymes on the 7th day was significantly higher (almost two-fold) as compared to the control group. PPO activity was detected on the 7th day after *Fomg* inoculation. Maximum PPO activity was measured in *P. aeruginosa* (P07-1) challenged with *Fomg*. The level of CAT activity was much lower than those of POX and PPO (**Fig. 1**). A great induction of proline was detected in eggplants treated with *P. aeruginosa* (P07-1, 56K-3 and 85A-2) at 7 days after pathogen inoculation; followed by *B. cereus* (B10a), *P. putida*

(P11-4), *Pseudomonas* sp. (P48-2) and *B. amyloliquefaciens* (76A-1) (**Fig. 1**). The application of the *Pseudomonas–Bacillus* mixture did not yield greater enzyme activities and metabolites than the application of PGPR alone.

The present study based on the ability of 10 PGPR isolates alone and in combination (*Pseudomonas–Bacillus*) significantly reduced the incidence of Fusarium wilt disease compared to non-bacterized plants. The protection mechanism seems to be the result of an antagonism between PGPR and *Fomg* and the induction of host-plant defense responses. In plants, reactive oxygen species (ROS) are associated with the response to pathogen attacks. Induction of plant defense can be triggered with the increase of antioxidant and phenolic enzymes which eventually lead to the accumulation of phenolic compounds (6). For example, Raju et al. (35) reported that the induction of proteins and accumulation of phenolics restricted *F. oxysporum* f. sp. *ciceris*. It is possible that higher levels of protein content after inoculation with biocontrol organisms may be related to the defense mechanisms of SAR or ISR that confer resistance to Fusarium wilt disease (41).

The results of our study showed that biotic inducers (*Pseudomonas* and *Bacillus*) resulted in greater accumulation of proline and antioxidant enzymes. Similarly, Moradi et al. (30) reported that PGPRs resulted in the accumulation of PR proteins via increased synthesis of β-1,3-glucanase and chitinase. *P. aeruginosa* (P07-1, 85A-2, 56K-3), *B. amyloliquefaciens* (76A-1), and *B. cereus* (B10a) resulted in increases of defense-related enzymes such as POX and PPO in eggplants. Similar results were also obtained from the work of Thangavelu et al. (39) in that they reported a two-fold increase in phenolic content along with the increase of PAL (phenylalanine ammonia lyase), chitinase, β-1,3-glucanase and POX in leaf tissues of banana three days after treatment with *Psedomonas fluorescens* strain Pf10 against *Fusarium oxysporum* f. sp. *cubense*. They suggested that the enhanced activities of defense enzymes and elevated content of phenolics might contribute to the bioprotection of banana plants against *F. oxysporum*.

POX and PPO have important roles in the formation of papilla that are located on the surface of cells and consist of various materials concentrated in the cell walls. This structure occurs as a response to host resistance through the production of hydrogen peroxide against pathogen penetration (22). Hydrogen peroxide is generally toxic to the microorganisms and also plays a role in strengthening cell walls with the biosynthesis of lignin through the activity of peroxidases. Similar findings with the above isolates were also found with CAT enzymes. There are various reports that these enzymes and metabolites are involved in the production or modulation of reactive oxygen species (ROS) which may play important roles directly or indirectly in reducing the pathogen spread and virulence (31). The increase in PPO activity is also associated with biosynthesis of lignin and other oxidative phenols and is also involved in triggering the hypersensitive reaction (HR).

Conclusions

There has been much research interest in PGPR and there is now increasing demand for commercialized PGPR products for many crops. These include the genera *Bacillus*, *Streptomyces*, *Pseudomonas*, *Agrobacterium*. Therefore, the new trend is to find new and efficient PGPR species or strains for the plants which have to grow under biotic or abiotic stress conditions. The present study showed that the application of PGPR to the rhizosphere of eggplant could increase the resistance of eggplant against to Fusarium wilt. The results of our study showed that biotic inducers (*Pseudomonas* and *Bacillus*) resulted in greater accumulation of proline and antioxidant enzymes. The accumulation of key defense enzymes, POX and PPO, and to a lesser extent CAT, by these bioagents most probably contributed to the induced resistance in eggplant against the pathogen. Since the promising isolates inhibited the growth of *Fomg* and reduced the severity of Fusarium wilt in eggplants through rapid colonization and antagonistic effects, it is possible to use these isolates in biocontrol strategies. The results obtained here could be used as a reference in the efforts to develop new control mechanisms such as application of combined abiotic and biotic inducers. In this case, the efficacy of the PGPRs could be enhanced via application of an abiotic elicitor in host-plant tissues. Genetic enhancement of PGPR strains to improve colonization and effectiveness might also play an important role for plant growth promotion. The use of multi-strain inocula of PGPR with known functions is of interest because they may offer great protection to multiple pathogens as well as to various harsh environmental conditions. The results obtained also encouraged us to evaluate the possible mode of action of these PGPRs in determining the expressions of pathogen-related (PR) proteins such as chitinase, glucanase and glucosidase, in which their expression levels and activities in the host tissue would help in developing new biocontrol strategies, including their concentrations and application time of PGPRs.

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