J. Agric. Sci. Mansoura Univ., 33 (6): 4145 - 4158, 2008

ROLE OF *Pseudomonas fluorescens* IN INDUCED SYSTEMIC RESISTANCE FOR TOMATO VASCULAR WILT DISEASE CAUSED BY *Fusarium oxysporum* F. SP. *LYCOPERSICI*

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ABSTRACT

Using an isolate of *Pseudomonas fluorescens* (*Pf*) in biological control was found to protect tomato plants from vascular wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* (*Fol*). In spite of the *In vitro* partially inhibition effect of *Pf* on mycelial growth of *Fol* by 47%, it found that *Pf* treatment one week before inoculation can decrease the diseases severity in tomato plants by 87%. These results indicating that systemic protection offered to tomato roots by the *Pf* for vascular wilt disease caused by *Pol* infection and explaining that ISR is involved.

Induction of defense enzymes by *Pf* against challenge inoculation with *Fol* in tomato was studied. Activities of phenylalanine ammonia-lyase (PAL), peroxidase (POX) and polyphenol oxidase (PPO) increased in bacterized tomato root tissues challenged with the pathogen. Analysis revealed that a PAL, POX and PPO were expressed at higher levels in bacterized tomato root tissues challenge inoculated with the pathogen. Activities of PAL and POX reached maximum at the 4th day while, activity of PPO reached maximum at the 6th day after challenge inoculation. Induction of these enzymes activities was found, not only in *Pf* treated root tissues challenged with the pathogen, but also in roots treated with *Pf* alone. These results suggest that induction of defense enzymes involved in phenylpropanoid pathway might have contributed to restriction of invasion of *Fol* in tomato plants.

Keywords: *Fusarium oxysporum* f. sp. *lycopersici*, induced systemic resistance, *Lycopersicum esculentum*, plant growth-promoting rhizobacteria, *Pseudomonas fluorescens*.

INTRODUCTION

Vascular wilt disease of tomato (*Lycopersicon esculentum* Mill.) caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) W.C. Snyder & H.N. Hansen (*Fol*) is one of the most economically important and widespread diseases of the cultivated tomato (Reis *et al*., 2005).

Environmental hazards and health concerns, associated with the use of chemical pesticides have resulted in an increasing interest in biological control as a promising alternative or a supplemental way of reducing the use of pesticides. Biological control involving the use of such plant beneficial microorganisms for plant protection such as bacteria and fungi, which naturally occurring in soil, is being considered as a viable substitute to reduce the use of chemical pesticides (Compant *et al*., 2005).

For over 100 years, scientists and naturalists have observed that when plants survive pathogen infection they develop an increased resistance to subsequent infections. In 1933, Chester reviewed 200 publications describing a phenomenon he termed physiological acquired immunity (Chester, 1933). In 1961, Ross demonstrated, in tobacco plants, that infections of TMV were restricted by a prior infection. This resistance was effective against not only

TMV but also tobacco necrosis virus and certain bacterial pathogens. Ross coined the term "systemic acquired resistance" (SAR) to refer to the inducible systemic resistance (Ross, 1961).

Root-colonizing bacteria that have a beneficial effect on plants are termed plant growth-promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1978). In the biological control of soil borne diseases of plants there is a form of induced resistance associated with the colonization of plant roots by certain PGPR, referred to as "induced systemic resistance" (ISR) (van Loon *et al.*, 1998). Results of several investigators showed that ISR is like to SAR in effective against a broad range of diseases caused by viruses, bacteria, and fungi (Wei *et al.*, 1996; van Loon *et al.*, 1998; Raupach and Kloepper, 1998, 2000; Murphy *et al.*, 2000; Nandakumar *et al.*, 2001; Zehnder *et al.*, 2001 and Niranjan Raj *et al.*, 2003).

Treatment with PGPR and fungi have been reported to sensitize plants to defend themselves against pathogen attack by triggering various defense mechanisms including production of phytoalexins, synthesis of phenolics (M'Piga *et al.*, 1997 and Chen *et al.*, 2000), accumulation of pathogenesis-related proteins (Meena *et al.*, 1999). The defense gene products peroxidases (POX) and polyphenol oxidases (PPO) catalyze the formation of lignin and phenylalanine ammonia-lyase (PAL) is involved in the synthesis of phytoalexins and phenolics (Karthikeyan *et al.*, 2005).

Pseudomonas spp. can induce systemic biochemical and ultra structural changes in the roots that lead to a greater ability of the host plant to defend itself against root infecting pathogens. The use of Fluorescent pseudomonads for controlling soil borne plant diseases has been well documented (Weller and Cook, 1986 and Sarma *et al.*, 2000).

Fluorescent *Pseudomonas* spp. are among the most effective rhizosphere bacteria in reducing soil-born diseases in disease-suppressive soils (Weller, 1988). These bacteria can antagonize soil borne pathogens through various mechanisms (*cf.* van Loon *et al.*, 1998). However, Wei *et al.*, (1991) suggested that ISR mediated by *Pseudomonas* spp. in several plant diseases interactions; neither competition nor antibiosis is involved in disease suppression.

The present study was carried out to assess the mode of action of non-pathogenic plant growth promoting rhizobacteria (PGPR) *Pseudomonas flourescens* in the management of *Fusarium* vascular wilt disease of tomato. The induction of defense enzymes related to the phenylpropanoid pathway known to be involved with induced systemic resistance such as phenylalanine ammonia-lyase, peroxidase and polyphenol oxidase in tomato vascular wilt disease caused by *Fusarium oxysporum* f. sp. *lycopersici* in response to application by PGPR biocontrol agent, *P. fluorescens* was investigated.

MATERIALS AND METHODS

Pathogen isolation:

Naturally infected tomato plants showing wilt symptoms were obtained in April 2006 from a commercial field growing in Tanta district, El-Gharbia Governorate, Egypt. Isolation procedures of the causal pathogen were

carried out from infected roots. After washing in running tap water, surface sterilized with 1% sodium hypochlorite solution for two minutes and rinsed three times in sterilized water, then dried between sterilized filter papers. Small pieces were placed on potato dextrose agar medium (PDA) supplemented with 300 mg of streptomycin sulfate/L in Petri-dishes and incubated at 25 ± 2°C. Pure culture was obtained using single spore technique and maintained on PDA at 27°C. Isolated pathogen was preliminary identified as *Fusarium oxysporum* according to Booth (1971 & 1977) and Nelson *et al.* (1983) and confirmed as *F. oxysporum* f. sp. *lycopersici* (*Fol*) by Agricultural Botany Department, Faculty of Agriculture, Kafr El-Shikh University.

Inoculum Preparation and Inoculation:

Fol isolate was grown on PDA plates for two weeks. Conidia were removed from the media surface by pipetting 10 ml of sterile saline onto the plates and gently scraping the conidia off the media surface by using a sterile spatula. The conidia were then separated from mycelium fragments by pouring the suspension through a sterile piece of gauze into a sterile beaker. The concentration of conidia was adjusted to 10^8 conidia/ml with a hemocytometer.

Inoculation was performed by dipping the root ball of the seedlings during transplanting into the conidial suspension in a 500 ml beaker for 5 minutes. Roots were dipped for 5 minutes in a beaker containing a sterile saline without conidia for non-inoculated control treatment. After inoculation, plants were planted in 25-cm diameter plastic pots containing sterilized sandy soil, ten plants each.

Plant growth promoting rhizobacterium (PGPR):

The bacterial antagonistic agent *Pseudomonas fluorescens* (*Pf*) isolate as PGRP were obtained from the laboratory of Prof. Z. Klement, Department of Pathophysiology, Plant Protection Institute, Budapest, Hungary. The isolate was maintained on King's B (KB) medium (King *et al.*, 1954), and routinely subcultured. Some slants were maintained at 4°C, and the cultures were grown at 27°C. Culture of *Pf* was grown for 24 hr. at 27°C and harvested by washing the culture with sterile saline by using a sterile spatula. Cell concentration was adjusted to an optical density of 0.06 at 660 nm which was equal to 10^8 cfu. ml^{-1}.

Tomato plants bacterization:

Bacterization was performed into the seedling's flat pots with 200 ml of the *Pf* cell suspension/pot. Pots of control treatment were drenched with only sterile saline and maintained.

Plant materials:

Tomato cultivar Castle Rock which susceptible to *Fusarium* wilt, was used in this study. Seeds were surface sterilized by immersion in 1% sodium hypochlorite for 20 min and rinsed three times in sterile distilled water, and sown in a flat 25x40 cm pots contained autoclaved sandy soil and grown in a greenhouse at 22 ± 2 °C. After 3 weeks, one set of the seedling's flat pot was bacterized with the *Pf* as described above. Another one week later, uniform seedlings were transplanted in 25-cm diameter plastic pots containing sterilized sandy soil. Inoculation with *Fol* was made during transplanting. The

experimental plots were made of four pots with ten plants each. They were watered daily and fertilized twice a week with a standard nutrient solution according to Pharand *et al.* (2002).

Disease severity:

Disease symptoms were recorded every 3 days for three weeks after inoculation. Disease severity (DS) evaluation was based on the leaf, vascular discoloration and wilting using 0-4 scale according to Kesevan and Chounhury, 1977 (cf. Özgönen *et al.*, 2001). The scale used was: 0 = no wilting symptoms; 1 = plant slightly wilted, vascular discoloration only in main root region; 2 = plant moderately wilted, yellowing of old leaves, spreading of vascular browning; 3 = plant severely wilted, dying of all leaves except end leaves and 4 = dead plant, seedling entirely wilted. The disease severity was calculated using disease scale values.

Disease severity was determined using the following equation (Song *et al.*, 2004):

$$\text{Disease severity (\%)} = \frac{(\Sigma \text{ Scale} \times \text{number of plants infected})}{(\text{Highest scale} \times \text{total number of plants})} \times 100$$

Ten plants per treatment were used and variance analysis of the treatment effect on measured data was performed.

***In vitro* antagonistic effect of *P. fluorescens* on the *F. oxysporum* f. sp. *lycopersici*:**

A disc (5-mm-diameter) carrying mycelium of *Fol* was taken from the end margin of 7-day-old culture, and placed at the centre of a 9 cm diameter PDA plate between two parallel streaks of *P. fluroscencs* which were 6 cm apart. Plates with the fungal disc and without bacterial streaks were served as control. When the fungal growth of the control approached the edge of the plates, antagonistic effect was assessed by relating mycelial diameters on plates inoculated with bacteria to mycelial diameter on control plates and percentage inhibition were calculated. Data from ten plates were used for this calculation.

Biochemical analysis:

Sampling was carried out at 0, 2, 4, 6, 8, 10, 12 and 14 d after inoculation. A replicate of 3 plants were uprooted each time. Roots were washed thoroughly with tap water, dried with filter paper, and cut with scissors. From each sample, one gm frozen in liquid nitrogen, ground in a precooled mortar and pestle, suspended in 3 ml cold 0.1 M potassium phosphate buffer (pH 7.2) containing 2% polyvinylpolypyrrolidone (PVPP) and 0.1 mM EDTA. The homogenate was centrifuged at 8,000 × g for 20 minutes. The supernatant was used for determination of PPO and POX enzymes activity. For PAL determination, samples were homogenized in 3 ml of ice-cold 0.1 M sodium borate buffer (pH 7.0), and the same proceeding was conducted.

Phenylalanine Ammonia-lyase (PAL) activity:

Activity of PAL (EC 4.3.1.5) was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid as described by Dickerson *et al.*

(1984). A sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8, and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30°C. The optical density value was recorded at 290 nm and the amount of trans-cinnamic acid formed calculated using its extinction coefficient of 9.63 mM^{-1} cm^{-1} (Dickerson *et al.*, 1984). Enzyme activity was expressed as μmol trans-cinnamic acid min^{-1} per gram fresh weigh.

Peroxidase (POX) activity:

Activity of POX (EC 1.11.1.7) was determined spectrophotometrically according to Zheng and van Huystee (1992). The reaction mexture (4ml) contained 10 mM sodium phosphate buffer (pH 6), 3.8 mM H_2O_2 as a substrate, 20 mM guaiacol, and 0.2 ml plant extract. After incubation at 30 ^{0}C for 10 min, the assay was determined by following the increase of absorbance at 470 nm. (extinction coefficient = 26.6 mM^{-1} cm^{-1}). Peroxidase activity was expressed as unit min^{-1} per gram fresh weigh.

Polyphenol oxidase (PPO) activity:

Activity of PPO enzyme ((EC 1.14.18.1) was measured spectrophotometrically by determining the decrease of the absorbency of yellow-color duo to the oxidation of Catichol at 495 nm. (Sadasivam and Manickam, 1992). The reaction mixture was 2.4 ml of 0.1 M Phosphate buffer (pH 6.5), 3 ml of Catichol solution (10 mM) and 0.3 ml plant extract. Changes in absorbance was recorded every 30 sec up to 5 mn. Calculation of the activity was carried out and illustrated as unit min^{-1} per gram fresh weigh.

Experimental treatments:

The study was conducted with 4 treatments of tomato plants as follow: (i) inoculation with *F. oxysporum* f. sp. *Lycoprcisi*, (ii) bacterization of plants by *P. fluorescens*, (iii) bacterization by *P. fluorescens* and inoculation with *F. oxysporum* f. sp. *Lycoprcisi* and (iv) neither infested with *Fol* nor bacterized with *Pf* as absolutely control treatment. Each treatment was replicated three times.

Statistical analysis:

All the data were analyzed by analysis of variance (ANOVA) with factorial treatment structure. When F values were significant at p>0.05, differences among the treatments were determined by Student test.

RESULTS

Isolation and identification of the causal pathogen:

Fungal isolate were isolated from naturally diseased tomato plants and showing wilt symptoms and identified as *Fusarium oxysporum* according to Booth (1971 & 1977) and Nelson *et al.* (1983) and confirmed as *F. oxysporum* f. sp. *lycopersici* by Agricultural Botany Department, Faculty of Agriculture, Kafr El-Shikh University.

Effect of PGPR (*P. fluorescens*) on Disease severity:

By applying *Pf* one week before inoculation with the *Fol* pathogen, disease incidence was significantly lower than inoculated and unbacterized treatment. Results showed that disease severity was 13.33%, however inoculated and not bacterized treatment recorded 83.3% (Fig 1).

***In vitro* antagonistic effect of *P. fluorescens* on the *F. oxysporum* f. sp. *lycopersici* radial growth:**

P. fluorescens was inhibited mycelial growth of *Fol* in vitro. Results obtained (Fig. 2) show that inhibition of Fol by 47%. Indeed, the use of *Pf* partially halted mycelial growth of *Fol* significantly.

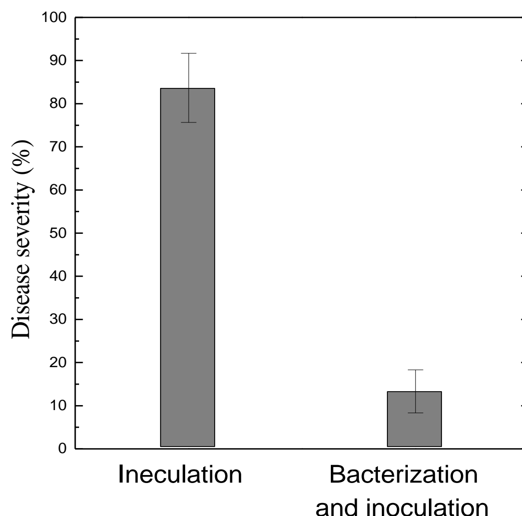

Fig. 1. Disease incidence of tomato plants inoculated with *F. oxysporum* f. sp. *lycopersici* and bacterization with *Pseudomonas fluorescens* one week before the inoculation.

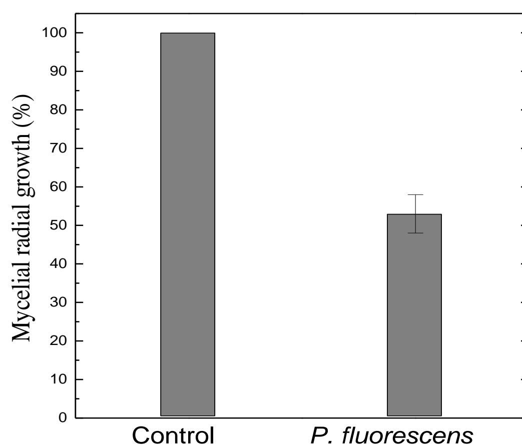

Figure 2. Effects of *Pseudomonas fluorescens* application *in vitro* on the mycelial radial growth of *Fusarium oxysporum* f.sp *lycopersici*.

Enzyme activities:

Activity of phenylalanine ammonia-lyase (PAL) (EC 4.3.1.5):

P. fluroscencs as a PGPR induced significant activity of PAL in the treated tomato roots over the period of time, however, in the untreated plants did not show any change in the pattern of PAL production (Fig. 3).

In the challenge-inoculated plants by *Fol*, the enzyme induction was highest on 4 days after challenge. Non-bacterized plants showed slightly higher activity of PAL upon challenge. The increase was significant compared to control on the 4th day of challenge.

There was highly significant increase in induction of PAL in the bacterized tomato leaves compared to the untreated. Even though the level started gradually declining after reaching a peak on 4th day, there was significantly increased activity of the enzyme by PGPR even on 14th day of bacterization. The highest activity of PAL upon inoculation by *Fol* was observed in the non- bacterized plants, which was significantly different than the bacterial treated plants. For all treatments, the peak was on the 4th day of challenge and then started diminishing.

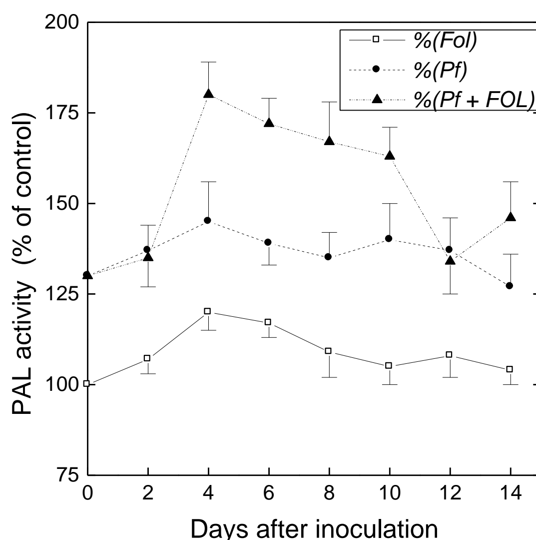

Figure 3. Phenylalanine ammonia-lyase (PAL) activity in roots of tomato plants cv. Castle Rock inoculated with *Fusarium oxysporum* f.sp *lycopersici*, bacterized by *Pseudomonas fluorescens* and challenged inoculation one week after bacteriazation. The PAL activity in the control plants was 4.8 ± 0.5 µmol trans-cinnamic acid g^{-1} FW min^{-1}. Means of three replicates are shown.

Activity of peroxidase (POX) (EC 1.11.1.7)

Activity of POX was increased significantly over time upon root bacterization (Fig. 4). Significantly higher activity was observed on the bacterization treatment. There maintained a higher level of POX in bacterized roots than in the non-bacterized. In the challenge inoculated plants, pathogen elicitors increased the POX level in tomato roots and a maximum level was reached on 4th day of challenge. All the treatments showed the highest peak on 4th day. The increase in enzyme activity induction was only significant in the 4th day in inoculated and non-bacterized plants. POX level diminished uniformly in all treatments immediately after 4th day of challenge.

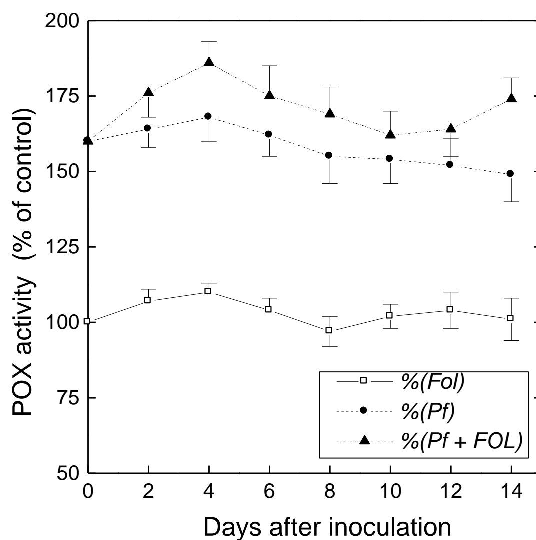

Figure 4. Peroxidase (POX) activity in roots of tomato plants cv. Castle Rock inoculated with *Fusarium oxysporum* f.sp *lycopersici*, bacterized by *Pseudomonas fluorescens* and challenged inoculation one week after bacteriazation. The POX activity in the control plants was 7.2 ± 0.8 µunit g^{-1} FW min^{-1}. Means of three replicates are shown.

Activity of polyphenoloxidase (PPO) (E.C. 1.14.18.1):

Activity of PPO reached the highest peak in a later stage unlike the other two enzymes studied. The PPO activity upon bacterization of the tomato roots occurred only on the 6th day after inoculation (Fig. 5). Untreated plants did not show any significant fluctuations in PPO activity in the study period. PGPR maintained the PPO activity in a significantly higher level throughout. Even in the plants, which were challenge inoculated after bacterization, there was an increase in the PPO level in roots compared to the control.

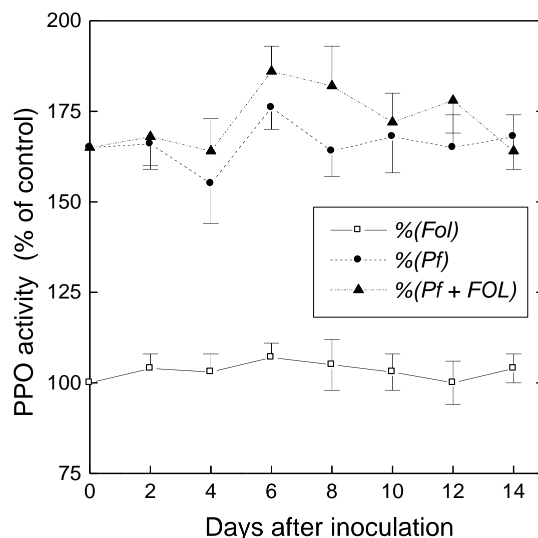

Figure 5. Polyphenoloxidase (PPO) activity in roots of tomato plants cv. Castle Rock inoculated with *Fusarium oxysporum* f.sp *lycopersici*, bacterized by *Pseudomonas fluorescens* and challenged inoculation one week after bacteriazation. The PPO activity in the control plants was 9.6 ± 0.9 µunit g^{-1} FW min^{-1}. Means of three replicates are shown.

DISCUSSION

Induced Systemic resistance (ISR) is potentiated by plant growth-promoting rhizobacteria (PGPR), of which the best characterized are strains within several species of *Pseudomonas* that cause no visible damage to the plant's root system (reviewed in van Loon *et al.*, 1998). As PGPR, *P. fluorescens* (*Pf*) has been demonstrated to induce systemic resistance for different diseases (Wei *et al*, 1991; Maurhofer *et al*, 1994 and Liu *et al*, 1995).

Results obtained (Fig 2), indicated that *In Vitro* test of *Pf* caused partially reduction of radial growth of *Fol* pathogen (47%). While, results illustrated in Fig. 1 showed that in greenhouse experiment, more significantly decreased of vascular wilt disease severity (87%) was obtained with *Pf* application. These results explained that, not only antagonistic effect of *Pf* for *Fol* control on tomato plants, but there is an ISR involved in this reaction. Bakker *et al.* (2007) reviewed that several investigators were obtained similar results.

In the present study, when tomato roots were bacterized with *Pf* there are some induced defence enzymes which in turn reflected in the reduced wilt symptoms when plants were challenge inoculated with the vascular wilt

pathogen *Fol*. Tomato roots showed increased synthesis of PAL, POX and PPO. Similar results of elevated levels of PAL, POX and PPO have been reported in cucumber plants infected with *Pythium aphanidermatum* and treated with PGPR strains (Chen *et al*., 2000).

The induction of defence related enzymes by PGPR treatment was correlated with the percentage vascular wilt suppression in the bacterized plants upon challenge inoculation with the fungal pathogen, *Fol*. The first enzyme of phenyl propanoid metabolism pathway is PAL, that catalyzes the trans-elimination of ammonia from L-phenyl alanine to form trans cinnamic acid which in turn enter different biosynthetic pathways leading to lignin (Hahlbrock and Grisebach, 1975). Increase in the PAL activity which observed in this study explains that the ISR in tomato plants bacterized *Pf* may be related to biosynthesis of lignin originate from L-phenyl alanine. Similar results were obtained by many investigators in several crops (Anderson and Guerra, 1985; van Peer *et al*, 1991; Daayf *et al*, 1997; Podile and lami, 1998 and Meena *et al*, 1999).

The present study revealed some increase in the POX activity due to *Pf* treatment and continues to be synthesized throughout the study period. POX is involved in lignification leading to disease resistance (Lagrimini *et al*, 1987). For lignification, specific cell wall peroxidases are thought to be required to generate H_2O_2 (Van Huystee, 1987). Polymerization of cinnamyl alcohols to lignin is catabolised by POX (Harkin and Obst, 1973). This is in agreement with Nandakumar *et al.* (2001), who published that ISR in rice has been correlated with an increase in activity of pathogenesis related peroxidase in PGPR treated plants inoculated with the rice sheath pathogens, *Rhizoctonia solani*.

The use of PGPR strains seems to be environmentally benign relative to current pesticides. These characteristics make ISR an attractive approach for managing crop pests in a sustainable manner within the scope of a conventional agriculture system. (Vallad and Goodman, 2004). *P. fluorescence* are capable to inducing higher levels of defence enzymes such as PAL, POX and PPO in tomato roots and it could be speculated that induced enzyme activities by *Pf* may be associated with the bio-synthesis of phenolic compounds and lignin that have been considered as major determinants in ISR against the vascular wilt disease. Further studies have to be performed on the ISR by PGPR to find out the exact signaling molecules involved in *Pf* mediated ISR in tomato plants.

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دور بكتريا سيدوموناس فلوريسينس فى المقاومة الجهازية المستحثة لمرض الذبول الوعائى فى الطماطم المتسبب عن فطر فيوزاريوم أكسيسبورام ليكوبيرسيسى

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وجد أن عزلة من بكتريا سيدوموناس فلوريسينس (*Pf*) فى حالة إستخدامها للمكافحة الحيوية كانت لها القدرة على زيادة حماية نباتات الطماطم من الإصابة بمرض الذبول الوعائى المتسبب عن الفطر فيوزاريوم إكسيسبورام ليكوبيرسيسى (*Fol*).

بالرغم من أن هذه البكتريا *Pf* كان لها تأثير جزئى، فى تجارب الأطباق، على تثبيط النمو الميسيليومى للفطر *Fol* بنسبة ٤٧ %، إلا أنه قد وجد أن معاملة النباتات بهذه البكتريا *Pf* قبل إسبوع واحد من التلقيح بالمسبب المرضى فإنه يمكنها أن تؤدى إلى خفض نسبة شدة الإصابة بالمرض فى نباتات الطماطم بحوالى ٨٧%. مما يثبت أن المقاومة للمرض عن طريق المعاملة بالبكتريا *Pf* تؤدى إلى إحداث مقاومة جهازية مستحثة فى أنسجة جذور نباتات الطماطم ضد مرض الذبول الوعائى الفيوزاريومى.

تم دراسة نشاط بعض إنزيمات المقاومة فى جذور نباتات الطماطم وتأثرها بالمعاملة بـ *Pf* وكذا عند عدوى النباتات بالمسبب المرضى *Fol*. أشارت النتائج إلى أن نشاط هذه الإنزيمات وهى: إنزيم فينيل آلانين أمونيالايز (PAL) , وأنزيم بيروكسيديز (POX) وكذلك إنزيم بوليفينول أوكسيديز (PPO) قد زادت فى أنسجة جذور نباتات الطماطم المعاملة بالبكتريا وكذا التى تم عدواها بالمسبب المرضى بعد المعاملة بالبكتريا. وقد وصل نشاط كل من إنزيمى PAL و POX وصلت إلى أعلا حد فى اليوم الرابع بعد العدوى. بينما وصل نشاط إنزيم PPO إلى أعلا حد فى اليوم السادس بعد العدوى. وقد وجد أن أعلا زيادة فى نشاط هذه الإنزيمات كان فى حالة جذور نباتات الطماطم التى تم عدواها بالمسبب المرضى بعد أسبوع من معاملتها بالبكتريا. وكانت الزيادة فى نشاط هذه الإنزيمات فى حالة جذور النباتات التى تم معاملتها بالبكتريا فقط دون عدوبالمسبب المرضى أقل مما سبق.

من النتائج المتحصل عليها يمكننا أن نوضح أن إستخدام هذه البكتريا *Pf* والذى يؤدى إلى زيادة نشاط هذه الإنزيمات والتى لها دور فى مسار إنتاج الفينيل بروبانويد يمكن أن يساهم فى مكافحة مرض الذبول الوعائى المتسبب عن فطر *Fol* فى نباتات الطماطم، أساساً عن طريق زيادة مقاومة النبات للمسبب المرضى. وأنه يمكن من الناحية التطبيقية إستخدام بكتريا *Pf* فى مكافحة المرض.