Phytopathol. Mediterr. (2003) 42, 268–274

Role of salicylic acid in *Pseudomonas aeruginosa* strain IE-6S$^+$-mediated induction of systemic resistance against *Meloidogyne javanica* in tomato

Imran A. Siddiqui and S. Shahid Shaukat

Soil Biology and Ecology Laboratory, Department of Botany, University of Karachi, Karachi-75270, Pakistan

Summary. Root colonization by certain non-pathogenic bacteria can induce systemic resistance to pathogen infections in plants. In a split-root assay with tomato plants, we investigated which determinants of the rhizobacterium *Pseudomonas aeruginosa* IE-6S$^+$ were important for induction of resistance to the root-knot nematode *Meloidogyne javanica*. *P. aeruginosa* IE-6S$^+$ produced 3.9±1.1 μg ml^{-1} salicylic acid (SA) in a liquid casamino acid medium under laboratory conditions. The bacterial inoculant induced resistance equivalent to the application of 10 mM synthetic SA. However, SA at this concentration did not produce significant mortality of *M. javanica* juveniles *in vitro*. Soil iron (2.4 mM $FeCl_3 \cdot 6H_2O$) did not markedly alter the resistance that *P. aeruginosa* IE-6S$^+$ induced in tomato roots, which suggested that *P. aeruginosa* IE-6S$^+$ activity was not iron-regulated. However, the resistance reaction was greatly enhanced when IE-6S$^+$ and SA were co-inoculated with 0.5% Tween-20. While IE-6S$^+$ colonized the tomato rhizosphere at 6.38 log cfu per g fresh weight of root during the first 3 days after inoculation, the bacterial populations declined steadily, reaching a mean population density of 4.73 log cfu g^{-1} fresh weight of root at 21 days. The bacterium was not isolated from the unbacterized half of the split root system.

Key words: induced systemic resistance, *Lycopersicon esculentum*, plant growth-promoting rhizobacteria, root-knot nematode.

Introduction

Interest in biological control has increased recently due to public concern about the use of chemicals in the environment in general. Studies on a number of plant-microbe interactions have shown that some antagonistic rhizobacteria protect plants from soil-borne pathogens either directly, by competition and antibiosis (Buchenauer, 1998) or indirectly, by induced systemic resistance (ISR) (Leeman *et al.,* 1995; Hasky-Günther *et al.,* 1998; Siddiqui and Shaukat, 2002). Rhizobacteria-mediated systemic resistance to control plant-parasitic nematodes is a fairly new research area, and studies on how to exploit this technique have started only recently. Studies on the mode of action of the rhizobacterium *Rhizobium etli* G12 on potato indicated that bacterium-free lipopolysaccharides (LPS) in this plant were in large part responsible for ISR to *Globodera pallida* infection (Reitz *et al.,* 2000), and that this resistance was independent of pathogenesis-related (PR) protein accumulation and did not increase peroxidase activity or enhance lignification of the potato tissue (Reitz *et al.,* 2001). On the other hand, in tomato roots infected with root-knot nematodes, several homologous plant-

Corresponding author: I.A. Siddiqui
E-mail: imran_75850@yahoo.com

defense genes, including peroxidases, chitinase, lipoxygenase, and proteinase inhibitor protein, are expressed locally within 24 h of inoculation with the nematodes (Lambert, 1995; Williamson and Hussey, 1996). Thus bacterial compounds which induce plant defense mechanisms against plant pathogens are highly variable.

One of these compounds, salicylic acid (SA) is an endogenous regulator of localized and systemic acquired resistance in many plants; when plants become infected, levels of SA increase to combat the infection. The exogenous application of SA in healthy plants will sometimes induce expression of the same set of defense-related genes that is induced in infected plants (Klessig and Malamy, 1994). Pseudomonads produce two siderophores, pyochelin and the precursor of pyochelin SA, which are thought to contribute to the protection of tomato plants from *Pythium* by *Pseudomonas aeruginosa* (Schroeter) Migula 7NSK2 (Buysens *et al.,* 1996). SA production has also been reported in *P. fluorescens* WCS374, WCS417r (Leeman *et al*., 1996) and CHA0 (Maurhofer *et al*., 1994) and this substance could be involved in ISR. Chen *et al.* (1999) demonstrated that higher SA levels enhanced the defence mechanisms of cucumber roots against the damping-off fungus *Pythium aphanidermatum*. Inoculation of the root tips of chickpea with *Pseudomonas fluorescens* strain 4-92 or with synthetic *o*-acetylsalicylic acid induced systemic resistance against the charcoal rot fungus *Macrophomina phaseolina* (Srivastava *et al.,* 2000). In contrast, some other studies indicated that SA does not play a role in the translocation of signals for ISR (Vernooij *et al.,* 1994; Pieterse *et al.,* 1996).

In our previous study (Siddiqui and Shaukat, 2002), the rhizobacterium *P. aeruginosa* IE-6S$^+$ was found to induce systemic resistance in tomato towards the root-knot nematode, *Meloidogyne javanica*. However, the mechanisms involved in *P. aeruginosa* IE-6S$^+$-mediated ISR are poorly understood. The objective of this investigation was to ascertain whether SA produced by *P. aeruginosa* biocontrol strain IE-6S$^+$ induces systemic resistance to *M. javanica* infection in tomato roots.

Materials and methods

Organisms, plants and culture conditions

Pseudomonas aeruginosa strain IE-6S$^+$ is a spontaneous streptomycin-resistant derivative of strain IE-6 and was originally isolated from the rhizosphere of sunflower grown in a sandy-loam soil in an agricultural field at Darsanochanoo, Pakistan (Siddiqui, 2002). For this study, the bacterium was routinely cultivated in liquid King's Medium B (KMB, King *et al.,* 1954) amended with 100 μg ml^{-1} of streptomycin on a rotary shaker (100 rpm) at 24°C for 48 h. Bacterial cells were harvested by centrifugation (2,800 *g*, 20 min), washed with sterile $MgSO_4$ (0.1 M) and resuspended in the same substance.

Egg masses of *M. javanica* (Treub.) Chitw., obtained from pure culture maintained on eggplant (*Solanum melongena* L.) roots were placed in sterile distilled water and incubated for 4–5 days at room temperature for hatching.

The tomato cultivar 'SUN 6002' (PVP) susceptible to *M. javanica* was used in the experiments. The seeds were surface-sterilized with 1% $Ca(OCl)_2$ for 3 min and planted in 35-cm-diam earthen pots containing steam-sterilized soil. Three-week-old seedlings were transplanted and used in the experiments.

SA production by *P. aeruginosa* IE-6S$^+$ *in vitro*

The bacterium was grown in casamino acid broth at 30°C for 24 h in the dark at 200 rpm. Subsequently, 100 μl of this culture was transferred to 25 ml of fresh medium and incubated as above for 36 h. The bacterial culture was centrifuged twice at 2,800 *g* for 15 min, the pellet discarded and the supernatant was filtered through two layers of Whatman No.1 filter paper. The filtrate was collected in a beaker and stored at 6°C in a refrigerator prior to use. Culture filtrate of *P. aeruginosa* was extracted with ethyl acetate (1:2) and the organic phase concentrated on a rotary vacuum evaporator (Eyela, Rikakiki Co. Ltd., Tokyo, Japan) under reduced pressure at 37°C. Qualitative analysis of SA was performed by TLC using fluorescent silica gel plates (F_{254}) eluted with acetic acid:chloroform (1:9, v:v). SA was detected under UV light and exposed to ammonia fumes, which gave rise to a blue colour. SA concentration was determined by adding 5 μl of 2 M $FeCl_3$ and 3 ml of water to 1 ml (from 2 mg of gummy extract dissolved in 2 ml ethyl acetate) of concentrated extract. The absorbance of the purple iron-SA complex, which developed in the aqueous phase, was

measured at 527 nm and compared with a standard curve of SA (Aldrich Chemical Co., Milwaukee, WI, USA) dissolved in ethyl acetate (De Meyer and Höfte, 1997).

Nematicidal activity of synthetic SA *in vitro*

To determine the effect of SA on *M. javanica*, 1 ml of 10 mM SA was put in a cavity glass slide to which a 1-ml suspension of freshly hatched, surface-sterilized juveniles (containing 40–50 juveniles ml^{-1}) was added. A glass cavity slide containing 1 ml sterile distilled water served as negative control. The treatments and the controls were replicated five times and kept at room temperature (28±2°C). After a 24-h incubation period, the dead juveniles were counted and percentage mortality was calculated. Nematodes were considered dead if they did not move when probed with a fine needle (Cayrol *et al.,* 1989). The experiment was repeated twice.

Split-root system

The split-root (two-pot) system allows the inoculation of the bacterium and nematode at separate locations on the root system (Hasky-Günther *et al.,* 1998). Three-week-old tomato seedlings were uprooted, washed with tap water, and their roots split into two halves with a sterilized dissecting scalpel. Each half of the root system was transplanted into a separate 7.5-cm-diam plastic pot containing 350 g soil per pot. Pots were attached to each other on the outside with a masking tape. A 10-cm-long, 2-mm-diam steel rod placed upright between the pots provided support for the plant. One half of the root system of each plant was treated with: i) 35 ml cell suspension (10^8 cfu ml^{-1}) of *P. aeruginosa* IE-6S$^+$; ii) 10 mM synthetic SA; iii) 2.4 mM $FeCl_3 \cdot 6H_2O$ (9.9 μg Fe^{3+} per g dry soil); iv) 0.5% Tween-20; v) IE-6S$^+$ suspension plus 2.4 mM $FeCl_3 \cdot 6H_2O$; vi) IE-6S$^+$ suspension plus 0.5% Tween-20; vii) 10 mM SA plus 2.4 mM $FeCl_3 \cdot 6H_2O$; or viii) 10 mM SA plus 0.5% Tween-20. Soil treated with 35 ml sterile $MgSO_4$ (0.1 M) served as controls. One week after seedling establishment, the other (un-bacterized) half of the root system was inoculated with 2000 freshly hatched juveniles of *M. javanica*. The experiment was a randomized complete block design with 5 replications, each consisting of one plant. Twenty-one days after inoculation, the root system was carefully removed, rinsed several times with tap water and blotted dry. The root samples were then boiled in 0.25% lactic acid fuchsin, and after homogenization in an electric grinder the penetrated juveniles were counted under a stereomicroscope. The experiment was performed twice.

Isolation of IE-6S$^+$ from the rhizosphere

To test whether bacteria moved from the bacterized half of the roots to the unbacterized half, in a separate experiment, isolation of IE-6S$^+$ was attempted from the rhizosphere of both halves of the root system. The experimental design was a 2×5 factorial with three replications, each consisting of one plant. The factors comprised 2 bacterial applications (with/without bacterium) and 5 nematode harvesting dates (0, 3, 7, 14 and 21 days after inoculation). *P. aeruginosa* IE-6S$^+$ and *M. javanica* were introduced into the soil as outlined above. The rhizosphere density of *P. aeruginosa* IE-6S$^+$ was determined by placing the roots with adhering soil in a 100 ml Erlenmeyer flask containing 10 ml of 0.1 M $MgSO_4$ solution (pH 6.5) plus 0.02% Tween-20. Ten-fold serial dilutions of the suspension were prepared and 100 ml aliquots from the appropriate dilutions plated on KMB supplemented with 100 mg ml^{-1} streptomycin.

Statistical analysis

Data were analyzed and subjected to one-way analysis of variance (ANOVA) using STATISTICA (Ver. 5.0; Statsoft Inc., Tulsa, OK, USA). Statistical significance was judged at the level of $P<0.05$. When the analysis was statistically significant, Duncan's multiple range test was performed to separate the means. The bacterial population was transformed to $\log_{10}(x+1)$ prior to analysis. An F-test was conducted to determine whether results from different experiments varied and whether pooling of data sets was warranted.

Results

SA production by *P. aeruginosa* IE-6S$^+$ and nematicidal activity of synthetic SA *in vitro*

SA in the culture filtrate of the *P. aeruginosa* IE-6S$^+$ strain was detected in TLC as a blue spot at an *Rf*-value of 0.91 after exposure to ammonia fumes. *In vitro* SA production by *P. aeruginosa* IE-6S$^+$, determined spectrophotometrically, revealed

that the bacterial inoculant cultivated in casamino acid liquid medium synthesized SA at 3.9±1.1 μg ml^{-1}. Synthetic SA was not toxic when tested *in vitro* against *M. javanica* juveniles (data not shown).

Split root trial

In the repeated trials, *P. aeruginosa* IE-6S$^+$ applied to one half of the split root system caused a significant systemic reduction (41%; $P<0.05$) in nematode penetration to the other half of the split root system (Fig. 1). Synthetic SA applied in the same manner also induced systemic resistance, reducing nematode penetration rates by 33% ($P<0.05$). The addition of $FeCl_3$ in the soil did not induce a resistance reaction by itself nor did it influence IE-6S$^+$ or SA activity against *M. javanica*. Soil application of Tween-20 alone did not reduce nematode invasion, but when Tween-20 was combined with *P. aeruginosa* IE-6S$^+$ or synthetic SA it enhanced the defence mechanism in tomato roots compared with the bacterium or SA alone. Tween-20 combined with IE-6S$^+$ reduced nematode invasion by 53%; combined with SA, by over 41%.

Plant colonization

To exclude direct antagonism between *P. aeruginosa* IE-6S$^+$ and *M. javanica*, systemic plant colonization by the bacterium was examined (Fig. 2). Inoculation of the bacterium resulted in 6.55 cfu of *P. aeruginosa* IE-6S$^+$ per g of dry soil. The bacterial inoculant colonized the tomato rhizosphere throughout the experiment, but its populations declined progressively. Three days after inoculation, bacterial populations in the rhizosphere were 6.38 log cfu per g fresh weight of the roots, which declined to around 4.73 log cfu per g fresh weight of root after 21 days. The bacterium was not isolated from the untreated half of the root system.

Discussion

An earlier study reported that *P. aeruginosa* strain IE-6S$^+$ caused a substantial reduction in *M. javanica* penetration and subsequent root-knot infection in tomato (Siddiqui and Ehteshamul-Haque, 2001). More recently, IE-6S$^+$ was found to impair infection from *M. javanica* indirectly by inducing systemic resistance (Siddiqui and Shaukat,

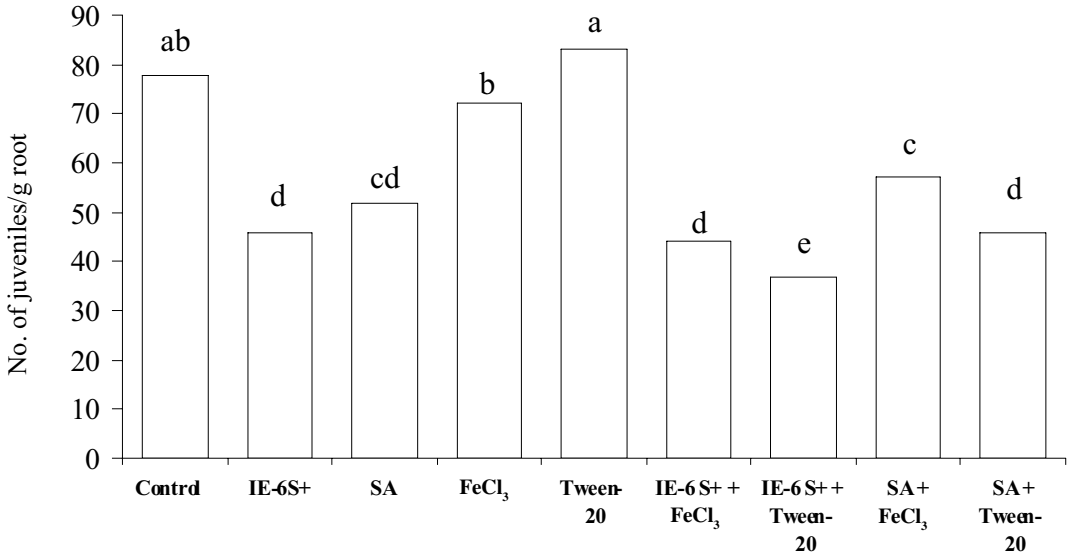

Fig. 1. The number of juveniles of *Meloidogyne javanica* that penetrated into the bacteria-free side of a split-root system. The other side was inoculated with either *Pseudomonas aeruginosa* (IE-6S$^+$); Salicylic acid (SA); Iron ($FeCl_3$); Tween-20; IE-6S$^+$ + $FeCl_3$; IE-6S$^+$ + Tween-20; SA + $FeCl_3$ or SA + Tween-20. (Mean values with different letters are significantly different according to Duncan's Multiple Range Test; $P<0.05$; n=10 obtained in two independent experiments each with 5 replicates).

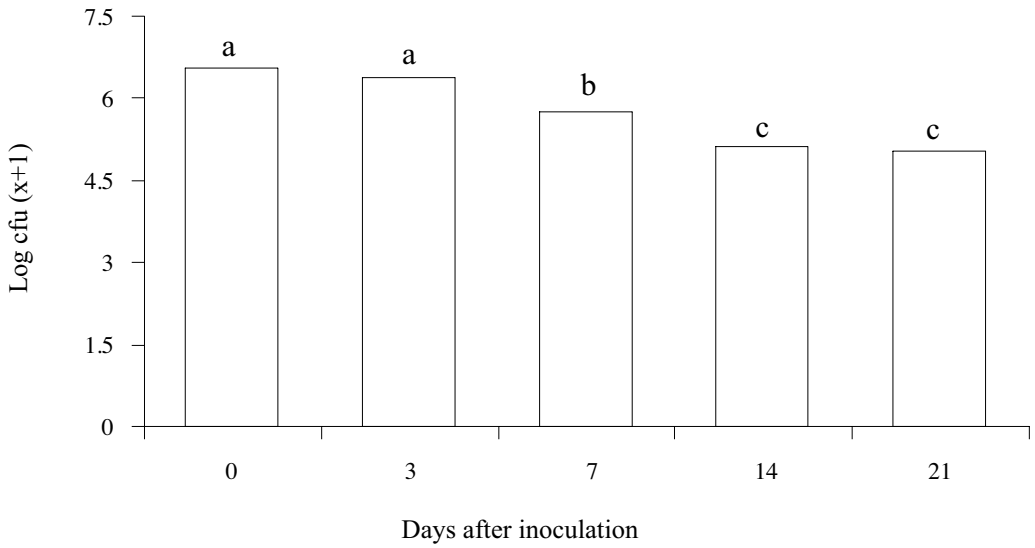

Fig. 2. Cfu of *Pseudomonas aeruginosa* strain IE-6S$^+$ isolated from the rhizosphere of the strain-treated half of the split root system. The bacterium was not isolated from the unbacterized half. Bars with the same letter are not significantly different at $P<0.05$ according to Duncan's multiple range test.

2002). Since preliminary experiments in this study showed that both a cell suspension and a cell-free culture filtrate of the strain enhanced the plant defence capacity of tomato roots, it was concluded that the bacterium produces some compound(s) that act as resistance inducing agent(s).

The idea that *P. aeruginosa* IE-6S$^+$ enhances the defence mechanism of tomato roots against *M. javanica,* and that this mechanism is in part triggered by the production of SA, is supported by several findings: i) *P. aeruginosa* IE-6S$^+$ synthesizes SA in casamino acid liquid medium *in vitro*; ii) synthetic SA at 10 mM does not cause significant mortality of *M. javanica* juveniles *in vitro* but it does reduce nematode invasion in tomato roots; iii) synthetic SA applied to one half of the root system significantly reduces nematode penetration in the other half of the root system, in a way similar to that of IE-6S$^+$; iv) direct antagonism between IE-6S$^+$ and the nematode is excluded because *P. aeruginosa* IE-6S$^+$ is not found in the rhizosphere of the non-bacterized half of the root system and, v) Tween-20, which increases SA uptake (Nandi *et al*., 2002), enhances the effect of both the bacterial cell suspension and synthetic SA against the nematode. In a recent study, SA (10 mM) sprayed on cowpea leaves inoculated with *M. incognita* reduced nematode infection and promoted plant growth (Nandi *et al*., 2002). That study also found that SA did not kill the nematodes *in vitro*, but SA sprayed on the plants induced the expression and accumulation of pathogenesis-related (PR)-1 protein in the leaves. Adding Tween-20 enhanced the effect of SA on the accumulation of PR-1 protein. The protein-findings contrast with Reitz *et al*. (2001), however, who reported that the resistance reaction triggered by *R. etli* G12 against *G. pallida* was not accompanied by higher levels of PR proteins such as chitinase and β-1,3-glucanase.

Nonetheless, a number of facts suggest that SA produced by bacteria is not the factor inducing systemic resistance in tomato against *M. javanica*: i) bacteria-derived SA has not been detected in the tomato rhizosphere or possibly, the root tissue; ii) iron-independent bacteria-mediated induction of systemic resistance is possible, and iii) there is no suitable control such as an SA-negative mutant. De Meyer and Höfte (1997) demonstrated that SA production by *P. aeruginosa* 7NSK2 was necessary to induce systemic resistance to *Botrytis cinerea* in bean. This was confirmed by the fact that the two SA-deficient mutants failed to induce resistance

to *B. cinerea*. By contrast, Press *et al.* (1997) suggested that SA produced by *Serratia marcescens* 90-166 was not important for inducing systemic resistance in cucumber and tobacco because SA-negative mutants of that strain induced the same resistance in cucumber, wild type tobacco, and *NahG*-tobacco (the *NahG* gene encodes the enzyme salicylate hydroxylase, which degrades SA), expressing salicylate hydroxylase as the wild type.

Rhizobacteria produce antimicrobial compounds that reduce pathogen infection by inducing systemic resistance in plants, but whether these compounds are produced at effective concentrations at the infection site or on the surface of the roots remains unknown. If a microorganism is found to be antagonistic *in vitro*, it is further tested in the field. If these tests are also positive, it is assumed that the same mechanism(s) is responsible in both cases. However, there is no justification for such an assumption unless the resistance-inducing agent is detected at active concentrations in the rhizosphere. Activity *in vitro* is greatly dependent on the medium in which an organism is grown. It remains to be seen what role the culture medium plays in the production of SA *in vitro,* and whether the quality (isomer) and quantity produced *in vitro* correlate with that produced in the plant after infection. By varying the iron nutritional state of the bacterium at inoculation, De Meyer and Höfte (1997) found that SA production by *P. aeruginosa* 7NSK2 was an essential precondition for the induction of systemic resistance, and that bacterial activity was iron-regulated. Treatment of bean roots with *P. aeruginosa* 7NSK2 significantly reduced the number of spreading *B. cinerea* lesions on the first leaves. However, this was only observed when the bacterium was prepared from the low-iron KB medium, and not when it was grown on an iron-rich LB medium.

Compared with other rhizobacteria that induce systemic resistance, the *in vitro* SA production of 3.9 μg ml^{-1} by *P. aeruginosa* IE-6S^+ was intermediate between *P. aeruginosa* 7NSK2 (De Meyer and Höfte, 1997), *P. fluorescens* CHA0 (Meyer *et al.,* 1992), and *P. fluorescens* WCS417r and WCS374 (Leeman *et al.,* 1996), which produced 5.6, 2, 8, and 55 μg ml^{-1} of SA *in vitro* respectively. The production of 3.9 μg ml^{-1} SA by *P. aeruginosa* IE-6S^+ seems enough to enhance the resistance of tomato roots against *M. javanica*. How *P. aeruginosa* IE-6S^+ derived SA induces resistance remains to be elucidated. Systemic SA transport from roots to the leaves is one possibility, but bacterial SA can also induce signals for systemic resistance at root level.

Literature cited

Buchenauer H., 1998. Biological control of soil-borne diseases by rhizobacteria. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 105, 329–348.

Buysens S., K. Heungens, J. Poppe and M. Höfte, 1996. Involvement of pyochelin and pyoverdin in suppression of *Pythium*-induced damping-off of tomato by *Pseudomonas aeruginosa* 7NSK2. *Applied and Environmental Microbiology* 62, 865–871.

Cayrol J.C., C. Djian and L. Pijarowiski, 1989. Study on the nematicidal properties of the culture filtrate of the nematophagous fungus *Paecilomyces lilacinus*. *Revue de Nématologie* 12, 331–336.

Chen C., R.R. Belanger, N. Benhamou and T.C. Paulitz, 1999. Role of salicylic acid in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber roots. *European Journal of Plant Pathology* 105, 477–486.

De Meyer G. and M. Höfte, 1997. Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces resistance to leaf infection by *Botrytis cinerea* on bean. *Phytopathology* 87, 588–593.

Harborne J.B., 1973. *Phytochemical Methods*. Chapman and Hall, London, UK, 278 pp.

Hasky-Günther K., S. Hoffmann-Hergarten and R.A. Sikora, 1998. Resistance against the potato cyst nematode *Globodera pallida* systemically induced by the rhizobacteria *Agrobacterium radiobacter* (G12) and *Bacillus sphaericus* (B43). *Fundamental and Applied Nematology* 21, 511–517.

King E.O., M.K. Warth and D.E. Raney, 1954. Two simple media for the demonstration of pyocyanin and fluorescin. *Journal of Laboratory and Clinical Medicine* 44, 301–307.

Klessig D.F. and J. Malamy, 1994. The salicylic acid signal in plants. *Plant Molecular Biology* 26, 1439–1458.

Lambert K.N., 1995. *Isolation of genes induced early in the resistance response to* Meloidogyne javanica *in* Lycopersicon esculentum. Ph.D. Thesis, University of California – Davis, Davis, CA, USA.

Leeman M., F.M. den Ouden, J.A. van Pelt, F.P.M. Dirkx, H. Steijl, P.A.H.M. Bakker and B. Schippers, 1996. Iron availability affects induction of systemic resistance to Fusarium wilt of radish by *Pseudomonas fluorescens*. *Phytopahology* 86, 149–155.

Leeman M., J.A. van Pelt, F.M. den Ouden, M. Heinsbroek, P.A.H.M. Bakker and B. Schippers, 1995. Induction of systemic resistance against *Fusarium* wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. *Phytopathology* 85, 1021–1027.

Maurhofer M., C. Hase, P. Meuwly, J-P. Métraux and G. Défago, 1994. Induction of systemic resistance of tobacco

to tobacco necrosis virus by the root-colonizing *Pseudomonas fluorescens* strain CHA0: influence of the *gacA* gene and of pyoverdine production. *Phytopathology* 84, 139–146.

Meyer J.-M., P. Azelrandre and C. Georges 1992. Iron metabolism in *Pseudomonas*: Salicylic acid, a siderophore of *Pseudomonas fluorescens* CHA0. *Biofactor* 4, 3–27.

Nandi B., N.C. Sukual, N. Banerjee, S. Sengupta, P. Das, and P.S. Babu, 2002. Salicylic acid enhances resistance in cowpea against *Meloidogyne incognita*. *Phytopathologia Mediterranea* 41, 39–44.

Pieterse C.M., S.C.M. van Wees, E. Hoffland, J.A. van Pelt and L.C. van Loon, 1996. Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis related gene expression. *Plant Cell* 8, 1225–1237.

Press C.M., M. Wilson, S. Tuzun and J.W. Kloepper, 1997. Salicylic acid produced by *Serratia marcescens* 90-166 is not the primary determinant of induced systemic resistance in cucumber or tobacco. *Molecular Plant-Microbe Interaction* 10, 761–768.

Reitz M., S. Hoffmann-Hergarten, J. Hallmann and R.A. Sikora, 2001. Induction of systemic resistance in potato by rhizobacterium *Rhizobium etli* strain G12 is not associated with accumulation of pathogenesis-related proteins and enhanced lignin biosynthesis. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 108, 11–20.

Reitz M., K. Rudolph, I. Schröder, S. Hoffmann-Hergarten, J. Hallmann and R.A. Sikora, 2000. Lipopolysaccharides of *Rhizobium etli* strain G12 act in potato roots as an inducing agent of systemic resistance to infection by the cyst nematode *Globodera pallida*. *Applied and Environmental Microbiology* 66, 3515–3518.

Siddiqui I.A., 2002. *Use of growth promoting bacteria in the control of root-infecting fungi and root-knot nematode of crop plants*. Ph.D. Thesis, Department of Botany, University of Karachi, Pakistan.

Siddiqui I.A. and S. Ehteshamul-Haque, 2001. Suppression of root rot-root knot disease complex by *Pseudomonas aeruginosa* in tomato: the influence of different inoculum, nematode populations, moisture and other plant associated bacteria. *Plant and Soil* 237, 81–89.

Siddiqui I.A. and S.S. Shaukat, 2002. Rhizobacteria-mediated induction of systemic resistance (ISR) in tomato against *Meloidogyne javanica*. *Journal of Phytopathology* 150, 469–473.

Srivastava A.K., T. Singh, T.K. Jana and D.K. Arora, 2000. Induced resistance and control of charcoal rot in *Cicer arietinum* (chickpea) by *Pseudomonas fluorescens*. *Canadian Journal of Botany* 79, 787–795.

Vernooij B., L. Friedrich, A. Morse, R. Reist, R. Kolditz-Jawahar, E. Ward, S. Uknes, H. Kessmann and J. Ryals, 1994. Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. *Plant cell* 6, 959–965.

Willimason V.M., R.S. Hussey, 1996. Nematode pathogenesis and resistance in plants. *Plant Cell* 8, 1735–1745.

Accepted for publication: November 7, 2003