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Role of salicylic acid in systemic resistance induced by *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *ciceri* in chickpea

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Abstract

Selected isolates of *Pseudomonas fluorescens* (*Pf*1–94, *Pf*4–92, *Pf*12–94, *Pf*151–94 and *Pf*179–94) and chemical resistance inducers (salicylic acid, acetylsalicylic acid, DL-norvaline, indole-3-carbinol and lichenan) were examined for growth promotion and induced systemic resistance against *Fusarium* wilt of chickpea. A marked increase in shoot and root length was observed in *P. fluorescens* treated plants. The isolates of *P. fluorescens* systemically induced resistance against *Fusarium* wilt of chickpea caused by *Fusarium. oxysporum* f.sp. *ciceri* (*Foc*Rs1), and significantly ($P = 0.05$) reduced the wilt disease by 26–50% as compared to control. Varied degree of protection against *Fusarium* wilt was recorded with chemical inducers. The reduction in disease was more pronounced when chemical inducers were applied with *P. fluorescens*. Among chemical inducers, SA showed the highest protection of chickpea seedlings against wilting. Fifty two- to 64% reduction of wilting was observed in soil treated with isolate *Pf*4–92 along with chemical inducers. A significant ($P = 0.05$; $r = -0.946$) negative correlation was observed in concentration of salicylic acid and mycelial growth of *Foc*Rs1 and at a concentration of 2000 µg ml^{-1} mycelial growth was completely arrested. Exogenously supplied SA also stimulated systemic resistance against wilt and reduced the disease severity by 23% and 43% in the plants treated with 40 and 80 µg ml^{-1} of SA through root application. All the isolates of *P. fluorescens* produced SA in synthetic medium and in root tissues. HPLC analysis indicated that *Pf*4–92 produced comparatively more SA than the other isolates. 1700 to 2000 ηg SA g^{-1} fresh root was detected from the application site of root after one day of bacterization whereas, the amount of SA at distant site ranged between 400–500 ηg. After three days of bacterization the SA level decreased and was found more or less equal at both the detection sites.

Key words: *Pseudomonas fluorescens* – *Fusarium oxysporum* f. sp. *ciceri* – Salicylic acid – Disease severity – *Fusarium* wilt

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Introduction

In recent years, strains of *Pseudomonas* have been extensively used for plant growth promotion and disease control. Several mechanisms have been suggested for disease control of soilborne pathogens by *P. fluorescens* involves production of siderophores, HCN, ammonia, antibiotics, volatile compounds etc. or by competing with pathogens for nutrients or colonization space (Glick 1995). In most of the cases *P. fluorescens* trigger a plant-mediated resistance mechanism called induced systemic resistance (ISR; Pieterse *et al*. 1996; Sticher *et al*. 1997; Van Loon *et al*. 1998; Benhamou *et al*. 2000). Rhizobacteria-mediated ISR has been reported for bean, carnation, cucumber, radish, tobacco, tomato and the model plant *Arabidopsis thaliana*, and effective against different types of plant pathogens (Metraux *et al*. 1990; Kwack *et al*. 2002). ISR has many similarities to pathogen-inducible defense called systemic acquired resistance (SAR), which renders uninfected plant parts more resistant towards a broad spectrum of pathogens (Ryals *et al*. 1996; Sticher *et al*. 1997). ISR

occurs via a different metabolic pathway from that SAR, which involves salicylic acid (Hoffland *et al*. 1996; Pieterse *et al*. 1996; Vindal *et al*. 1998). Thus, the ISR signaling pathway clearly differs from the one controlling pathogen-induced SAR. The state of SAR is characterized by an early increase in endogenously synthesized salicylic acid (Metraux *et al*. 1990).

Salicylic acid (SA) is a phenolic compound that affects a variety of biochemical and molecular events associated with induction of disease resistance. SA has been shown to play an important role in expression of both local resistance controlled by major genes and systemic induced resistance developed after an initial pathogen attack (Hammerschmidt and Smith-Becker 2000). A question is often raised as to whether there are one or several SA signal transduction pathways mediating various defense or several signal related mechanisms. Application of exogenous SA at a concentration of 1 to 5 mM has been long known to induce pathogenesis-related (PR) gene expression and acquired resistance against a variety of microbial pathogens (Ward *et al*. 1991; Meena *et al*. 2001). Low concentrations (10 to 100 µM) of SA have also shown to be sufficient for pathogen-induced defense gene expression, H_2O_2 accumulation and hypersensitive cell death in plant suspension cultures (Levine *et al*. 1994; Kauss and Jeblick 1996). Many studies indicated that SA accumulation was associated with plant physiological responses to pathogen infection. Malamy *et al*. (1990) reported that SA level increased as much as 20 fold after TMV infection on resistant cultivar tobacco leaves. Exogenous SA was found to induce PR-protein accumulation in tobacco and this accumulation correlated with increased TMV resistance (Van Loon and Antoniw 1982). Some studies indicated that SA may not be a translocated primary signal for SAR, and SA may only play a regulatory role in the expression of SAR genes (Vernooji *et al*. 1994; Pieterse *et al*. 1996; Seah *et al*. 1996). They showed that SA may not be involved in all cases of systemic resistance and did not act as exogenous inducers against plant pathogens. In general, SA may play a more important role for SAR than for ISR. However, SA still is an uncertain factor as a signal for SAR or ISR. It is also not clear whether SA is produced in infected plants or by PGPR strains and what role does SA play in resistance to chickpea root disease.

Chickpea (*Cicer arietinum* L.) is one of the important pulse crops of the world after bean and pea and approximately 75% of the world's chickpea is produced by India alone (FAO 1993). However, chickpea productivity remained virtually stagnant over recent decades due to its susceptibility to some diseases such as wilt caused by *F. oxysporum* f. sp. *ciceri*, charcoal/collar rot caused by *Macrophomina phaseolina* and blight (*Ascochyta rabiei*). These pathogens cause serious diseases in chickpea which is wide spread in India, Iran, Pakistan, Nepal, Burma, Spain, Mexico, Peru, Syria and USA (Nene *et al*. 1989). More than 80% reduction in yield can occur, if measures are not taken to control these pathogens. At least 4 races of *F. o. ciceri* exist in India, and 7 races in the other regions of the world have been reported (Giri *et al*. 1998). Keeping these in view we have focused on the plant growth promoting and ISR activity of some isolates of *P. fluorescens* alone or in combination with chemical inducers in chickpea, with a special emphasis on the efficacy of resistance inducers for control of *F. o. ciceri*, and evaluation of SA produced by *P. fluorescens* isolates.

Materials and methods

Plant material, bacterial isolates and pathogen. Seeds of chickpea (*Cicer arietinum* L.) cultivar JG-62 (provided by Dr. R. P. Thakur, International Crops Research Institute for Semi-Arid Tropics, Patancheru, India) highly susceptible to *F. o. ciceri* were used for study. The seeds were washed with 2% soap solution (phosphate free liquid), soaked under running water for 30 min, surface sterilized in 2% sodium hypochlorite for 10 min, and rinsed in sterile distilled water (SDW) before sowing. Strains of *P. fluorescens* were isolated from the rhizosphere soil of chickpea from different area of Varanasi (India) and grown on KB medium. Isolation and identification was done according to the method as described earlier by Yeole and Dube (1997) and isolates of *P. fluorescens* were designated as *Pf*1–94, *Pf*4–92, *Pf*12–94, *Pf*151–94 and *Pf*179–94. The isolates were grown in KB medium in 500 ml Erlenmeyer flasks on a rotary shaker (50 rpm) for 24 h at 28 ± 2°C and cell concentration was adjusted to 10^8 cells ml^{-1}. The pathogen, *F. o. ciceri* Rs1 (*Foc*Rs1) was obtained from the Laboratory of Applied Mycology, Department of Botany, Banaras Hindu University, Varanasi, India.

Plant growth and disease induction treatment. Surface sterilized chickpea seeds were sown in plastic pots (20 cm diam., 3 seeds pot^{-1}) filled with *Foc*Rs1 infested and *P. fluorescens* inoculated soil. Inoculum of *Foc*Rs1 was prepared in sterile sand: maize meal medium (50 g + 1.5 g maize meal + 10 ml water) incubated for 15 days at 28 ± 2°C. The inoculum (5% w/w) was mixed thoroughly in double autoclaved sandy loam chickpea field soil, the isolates of *P. fluorescens* were poured separately (30 ml pot^{-1}) to the pots as per following treatments. (i) blank control – pots containing sterilized soil, (ii) *Foc*Rs1 control – pots containing soil infested with the pathogen, (iii) *P. fluorescens* treatment – the seeds sown in pots containing either of 5 isolates of *P. fluorescens* and (iv) the soil containing isolates

of *P. fluorescens* + *Foc*Rs1. Experiment was carried out as complete randomized designs (CRD) in a greenhouse.

Influence of resistance inducers. For infesting soil with *Foc*Rs1, pots (diameter 20 cm) were filled with 300 g of sterilized chickpea field soil (sandy loam), mycelial disc (5 cm diameter, 5 d old) of *Foc*Rs1 was added at the soil surface and covered with 50 g of soil. In case of control, the same amount of pathogen-free agar medium was added. The pots were watered and incubated for 5 days in the greenhouse before sowing chickpea seeds (3 seeds pot^{-1}). Aqueous solution (2 mM) of resistance inducers e.g. salicylic acid, acetylsalicylic acid, DL-norvaline, indole-3-carbinol and lichenan (Sigma Chemicals, USA) were prepared in deionized water and added to each pot (30 ml pot^{-1}). Similarly, in a separate experiment the cell suspensions of *Pf*4–92 (ca. 10^8 cell ml^{-1}) were also applied immediately after sowing the chickpea seeds (30 ml pot^{-1}). Likewise, to study the interaction of *Pf*4–92 and the various chemical inducers on the disease severity of chickpea, 15 ml of bacterial cell suspensions (10^8 cells ml^{-1}) and 15 ml of the chemical solutions were mixed and applied as soil drench.

Induced systemic resistance by P. fluorescens. A split root technique was used to observe the induced systemic resistance by *P. fluorescens* in green house. The roots of 10-day-old plants were split with a razor and transplanted into two plastic pots. The chickpea roots spread into two separate plastic pots. One week later, one side of the root system was bacterized with 1 ml of *P. fluorescens* (10^8 cells ml^{-1}) or treated with SDW. After three days, the other side of the root system was challenged with 1 ml of conidial suspension of *Foc*Rs1 (ca. 10^6 conidia ml^{-1}). Pots were covered with plastic bags to prevent pathogen contamination. Seedlings were watered daily with sterilized water (20 ml pot^{-1}). Appearance of disease symptoms (wilting) was checked every day for 27 days.

Effect of SA on mycelial growth. A Petri-plate test was carried out to observe the direct effect of SA on the growth of *Foc*Rs1. Actively growing *Foc*Rs1 (5 mm disc) was placed at the center of each SA-amended plates containing potato dextrose agar medium (PDA, pH 5.5). The SA concentrations in the medium were 0, 50, 100, 200, 500, 1000 and 2000 µg ml^{-1}. The diameter of colony on SA plate was measured 5 day after inoculation from the inoculum disc edge to the growing colony edge.

Effect of exogenous SA on root disease and growth promotion. Effect of exogenously supplemented SA on chickpea wilt was evaluated in hydroponics system. Polycarbonate growth chambers were fabricated as described by Yedidia *et al*. (2001). Surface sterilized chikpea seeds (2 seed box^{-1}) were placed on a sterile cotton gauze spread over a stainless steel wire mesh fixed 1 cm above the 200 ml plant growth medium (PGM, Yedidia *et al.* 2001) in bottom of the growth chambers. The boxes were incubated in an environmental chamber at 28 ± 2 °C, 80% RH, and 14h cool fluorescent light. The boxes were connected with sterilized polycarbonate tubes (5 mm dia.) for aeration. A continuous flow of air (50 ml min^{-1} approx.) filtered by 0.45 µ filter (Millipore), generated through a bubbler was maintained throughout the experiment. Ten-day-old seedlings were injected with 10µl of SA (pH 6.5) by sterile syringe at the base of stem at various concentrations of SA (0, 100, 200, 500, 1000 and 2000 µg ml^{-1}). In another set of experiment, the seedlings were treated with SA by adding 10 ml of each solution of the above concentrations to the boxes containing the nutrient solution so that the SA concentrations in each tube remain as 0, 4, 8, 20, 40 and 80 µg ml^{-1}. Five days after SA treatment, seedlings were inoculated with conidial suspension of *Foc*Rs1 (ca. 10^6 conidia ml^{-1}) by injecting 1 ml of the conidial suspension to the bottom of the box with a sterile syringe. Disease severity was examined for the next 27 days by the formula: % of DS = (Infection length in control – infection length in treatment) × 100 / Infection length in control. Shoot and root length of plants was measured before the plants were removed at 45 days.

Extraction of SA produced by P. fluorescens. Each *P. fluorescens* isolate was seeded in to plastic tube containing 20 ml KB broth and incubated at 28 ± 2 °C for 28 h and SA was extracted after 48 h. The liquid culture was centrifuged at 2800 g for 20 min at 4 °C and then supernatant was acidified to pH 2. The solution was filtered through nylon membrane under vacuum and partitioned twice with 2 ml $CHCl_3$, finally dried under nitrogen stream at 40 °C. Each sample was re-suspended in 1 ml of 23% methanol in 20 mM sodium acetate buffer (pH 5) and then the samples were analyzed with HPLC (Yalpani *et al.* 1991).

SA extraction from bacterized or induced chickpea root tissue. To evaluate the level of SA in roots bacterized by *P. fluorescens* isolates experiments were performed in hydroponics system as described earlier. Roots of 10-day-old seedlings were bacterized at the tip by dipping the roots in suspension of *P. fluorescens* cells (ca. 10^8 cells ml^{-1}) in 1% carboxy methyl cellulose. After bacterization seedlings were transposed to the boxes and incubated in an environmental chamber at 28 ± 2 °C, 80% RH, and 14 h cool fluorescent light. Root samples were taken at an interval of one day from the bacterization site and a distant site until 3 days. One gram of bacterized and non-bacterized roots was separately

homogenized with liquid nitrogen. The homogenate was rinsed with 2.5 ml of 90% methanol, sonicated and centrifuged at 2800 g for 20 min. The pellet was extracted again with 2 ml of methanol. The supernatants were dried using a stream of nitrogen. The residue was resuspended in 2 ml 5% trichloroacetic acid (TCA) and centrifuged at 2800 g for 15 min. The supernatant was partitioned twice with an extraction medium ethyl acetate : cyclopentane : isopropanol (100 : 99 : 1). The top phase was combined and dried under a nitrogen stream (Raskin *et al.* 1989). Residues were resuspended in 1 ml of 23% methanol in 20 mM sodium acetate buffer and the solution was passed through 0.2µm nylon membrane via a vacuum at 250 mm Hg.

SA was detected by HPLC at 280 nm with a Bondapak C18 column (3.9 mm × 30 cm), with a mobile phase flow rate at 0.5 ml min^{-1}. SA was separated isocratically with 23% methanol (v/v) in 20 mM sodium acetate buffer (pH 5). Ten µl of each sample was injected in to the column. Retention time of isolated SA was compared with standard SA (Sigma). The SA was estimated µg ml^{-1} bacterial culture and ng g^{-1} fresh root. All the experiments were repeated twice with 5 replicates for each treatment. Effect of SA on mycelial growth, disease severity and plant growth was subjected to linear regression analysis.

Results

Plant growth promotion and induced systemic resistance

A marked increase in shoot and root length was observed in *P. fluorescens* treated plants (Fig. 1). The effect was more pronounced in roots as compared to shoot. For example, 33% increase in root length was obtained in the plants treated with *Pf*4–92, whereas the increment was 10% in shoot length as compared to control. *Pf*151–94 was found least effective in growth promotion activity. In general, *Foc*Rs1 inhibited the root development when used in combination with *P. fluorescens* isolates or alone (Fig. 1). For example, 75% and 40% reduction in root length was recorded when the plants were grown in *Foc*Rs1 infested and *Foc*Rs1 plus *Pf*4–92 inoculated pots, respectively. The isolates of *P. fluorescens* systemically induced resistance against *Fusarium* wilt of chickpea caused by *F. o. ciceri*. The result exhibits that the isolates reduced the wilt disease by 26–50% compared to control. Maximum reduction (50%) in disease severity was observed with *Pf*4–92, followed by *Pf*1–94 and *Pf*151–94 (46%; Table 1). Seventeen to 45% increase in plant height was recorded in the plants treated with *P. fluorescens* isolates in split root system (Table 1).

Table 1. Split root challenge inoculation by different isolates of *Pseudomonas fluorescens*

P. fluorescens isolates	Disease severity (%)	Plant height (cm)
*Pf*1–94	22.3 ± 1.5	31.4 ± 4.5
*Pf*4–92	22.2 ± 2.1	35.5 ± 2.3
*Pf*12–94	24.0 ± 2.0	30.0 ± 2.5
*Pf*151–94	23.6 ± 2.5	29.0 ± 3.3
*Pf*179–94	25.0 ± 1.2	28.7 ± 2.3
Control	43.5 ± 5.1	24.4 ± 1.5

C. D. = 4.62 for disease severity and 3.83 for plant height, computed at $P = 0.05$; ± = S.D.

Effect of resistance inducers

Resistance inducers exhibited varied degree of protection to chickpea plant against *Fusarium* wilt disease (Fig. 2). Disease severity was maximum (52%) in *F. o. ciceri* control, whereas significant ($P = 0.05$) reduction of the disease was recorded when chemical inducers were applied alone or combinations with *P. fluorescens*. Out of five chemical inducers, SA showed the highest protection of chickpea seedlings against wilting. In general, 31–45% reduction in disease was recorded in plants treated with resistance inducers. Greater reduction in disease occurred when chemical inducers were applied in combination with different isolates of *P. fluorescens*. For instance, 52–64% reduction of wilting was observed in soil treated with *Pf*4–92 isolate along with chemical inducers (Fig. 2).

Effect of SA on mycelial growth

A significant ($P = 0.05$; $r = -0.946$) negative correlation was observed in concentration of salicylic acid and mycelial growth of *Foc*Rs1 in a Petri-plate assay. However, at 100 µg ml^{-1} concentration no inhibition was observed and at a concentration of 2000 µg ml^{-1} mycelial growth was completely arrested (Fig. 3).

Influence of exogenous SA on disease severity and growth promotion of chickpea

Influence of exogenously supplied SA in chickpea growth and wilt disease was studied under hydroponics system. In general, SA stimulated systemic resistance against *Fusarium* wilt by both the methods i.e. injection of SA in the stem of chickpea plants and also by root application (Tables 2, 3). Fifteen per cent reduction in disease severity was observed when SA is applied through stem injection at a concentration of 200 µg ml^{-1} whereas, the disease severity reduced by 40% at 2000 µg ml^{-1} (Table 2). In root application of SA, reduc-

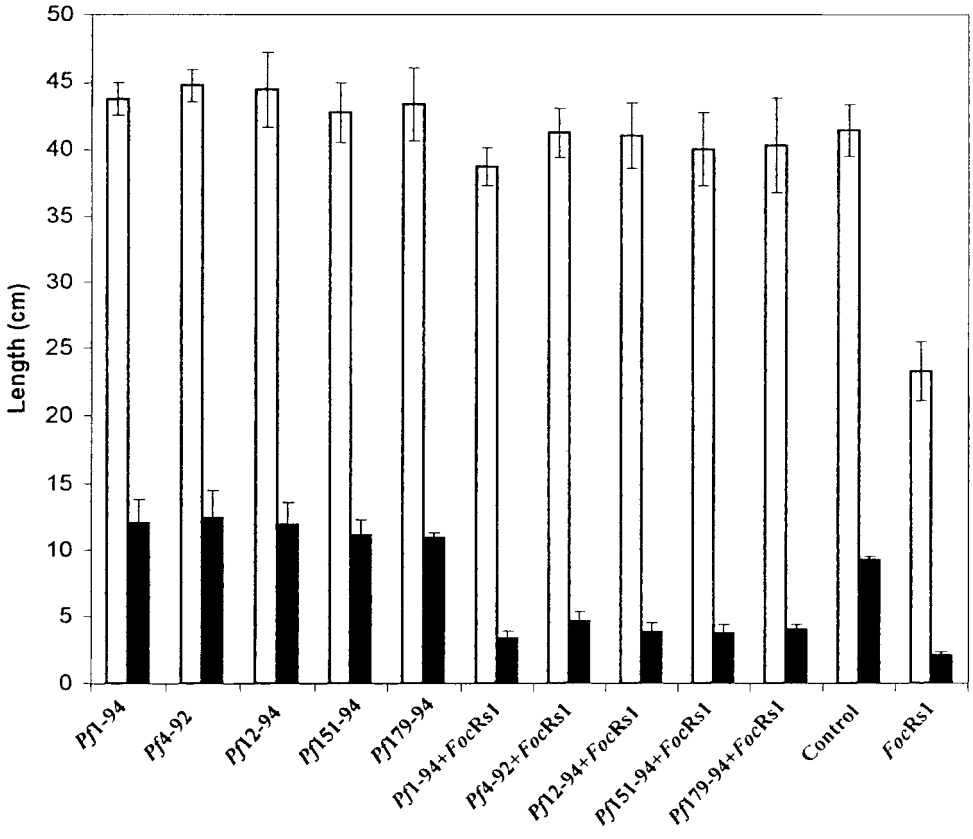

Fig. 1. Growth promotion of chickpea by different *Pseudomonas fluorescens* isolates. Bars represent: □ shoot length and ■ root length; | = S.D.

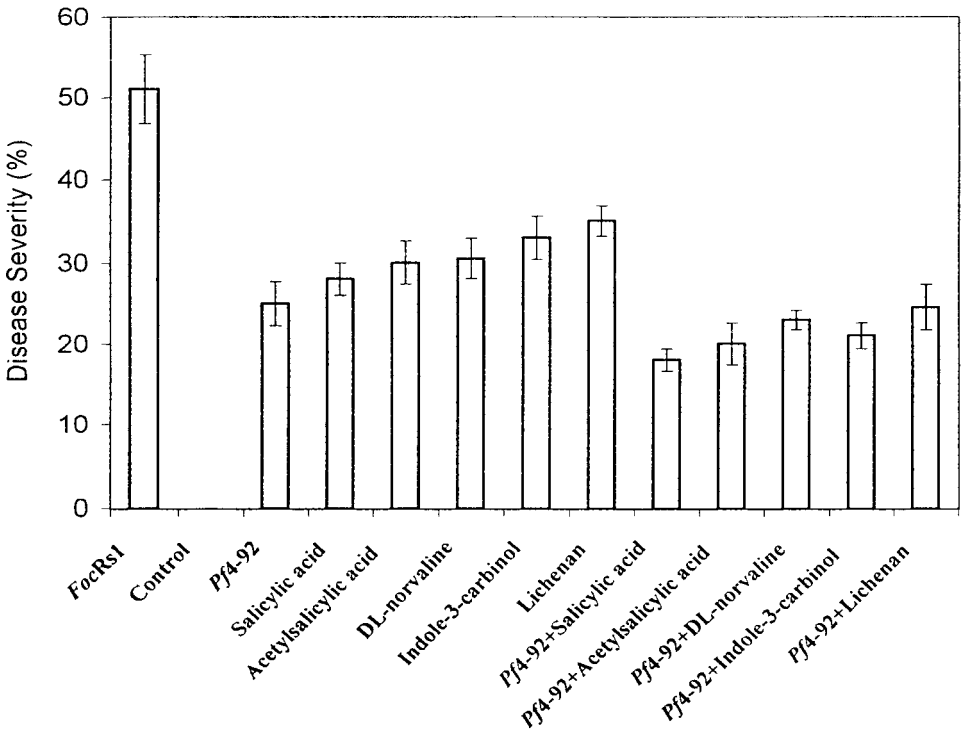

Fig. 2. Influence of *Pseudomonas fluorescens* (*Pf*4–92) and chemical resistance inducers on *Fusarium* wilt severity in chickpea. | = S.D.

Table 2. Influence of exogenous SA injected to plants on disease severity and growth promotion

SA concentration (µg ml^{-1})	Disease severity (%)	Shoot length (cm)	Root length (cm)
0	49 ± 2.4	20.0 ± 3 .5	7.4 ± 1.4
100	47 ± 4.1	24.3 ± 1.8	7.7 ± 1.1
200	42 ± 2.8	24.3 ± 1.5	10.0 ± 2.2
500	38 ± 1.6	24.6 ± 2.1	12.5 ± 1.9
1000	35 ± 2.0	25.0 ± 4.0	12.5 ± 2.1
2000	29 ± 1.7	26.4 ± 2.3	13.0 ± 2.3

C.D. = 4.15 for disease severity; 5.89 for shoot length; and 2.41 for root length computed at $P = 0.05$; ± = S.D.

Table 3. Influence of exogenous SA applied to roots on disease severity and growth promotion of chickpea

SA concentration (µg ml^{-1})	Disease severity (%)	Shoot length (cm)	Root length (cm)
0	43.0 ± 4.3	11.0 ± 2.9	8.0 ± 3.3
4	38.7 ± 3.0	12.6 ± 1.6	8.0 ± 2.3
8	35.0 ± 3.0	15.0 ± 2.9	9.0 ± 1.4
20	34.0 ± 2.6	19.0 ± 2.5	10.5 ± 1.2
40	33.3 ± 2.7	20.3 ± 1.2	12.0 ± 2.5
80	24.3 ± 1.3	21.0 ± 1.2	12.5 ± 1.6

C.D. = 3.12 for disease severity; 2.17 for shoot length; and 1.48 for root length, computed at $P = 0.05$; ± = S.D.

tion in disease severity was more pronounced. For instance, 23% and 43% reduction in disease was recorded in the plants treated with 40 and 80 µg ml^{-1} of SA through root application (Table 3). In both the treatment root and shoot length of plant significantly ($P = 0.05$) increased and a positive correlation ($r = 0.84$ to 0.95) was observed in concentration of SA and root and shoot length (Fig. 4 A, B).

Detection of SA by HPLC

All the isolates of *P. fluorescens* produced SA in synthetic culture medium and in root tissues. However, SA production was significantly greater in medium than in root tissues. HPLC analysis indicated that *Pf*4–92 produced comparatively more SA than the other isolates (Fig. 5). The level of SA in the roots of bacterized plants was estimated at two sites (Fig. 6). In general, from the application site of root 1700 to 2000 ηg SA g^{-1} fresh root was detected after one day of bacterization whereas, the amount of SA at distant site ranged between 400–500 ηg in same samples. After three days of bacterization the SA level decreased and was found more or less equal at both the detection sites. For example, 1250 and 1200 ηg SA g^{-1} fresh root was detected in *Pf*4–92 treated roots, and 1195 and 1190 ηg SA g^{-1} fresh root was detected in *Pf*12–94 treated roots at bacterized and distant sites, respectively (Fig. 6).

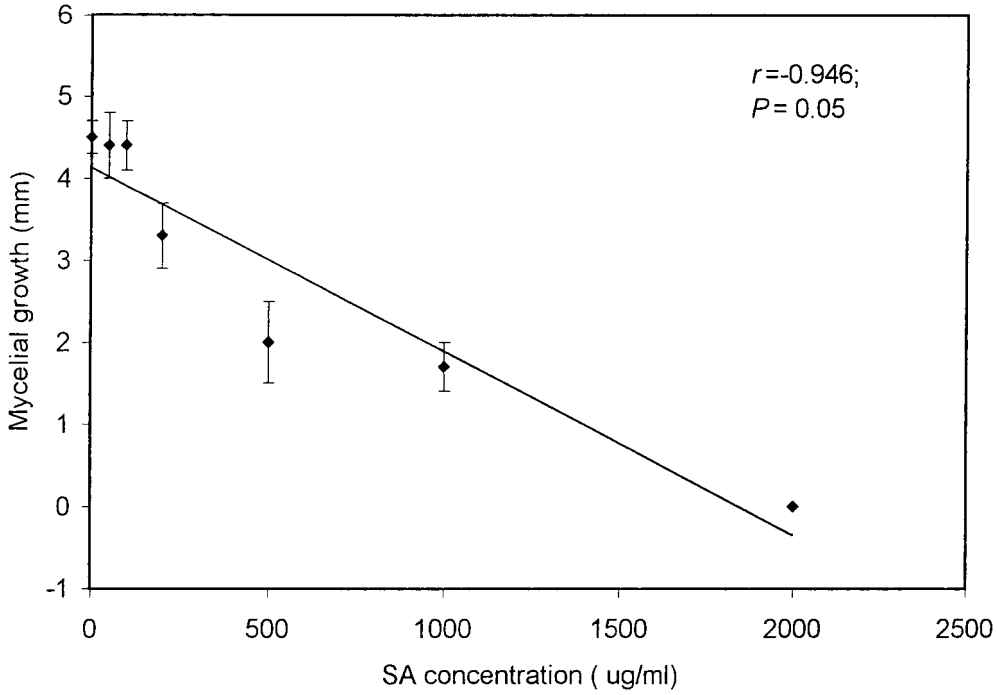

Fig. 3. Effect of salicylic acid (SA) on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* Correlation coefficient $r = -0.946$, $P = 0.05$; | = S.D.

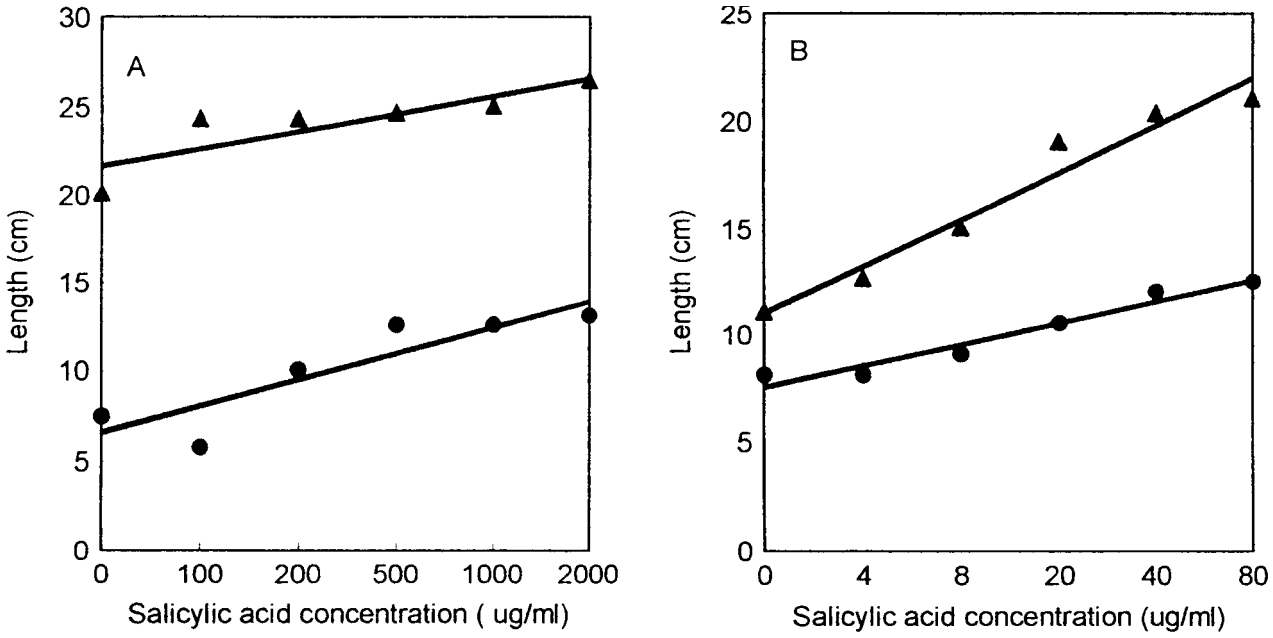

Fig. 4. Effect of salicylic acid on root and shoot length of chickpea plants. A = the plants injected with SA; correlation coefficient $r = 0.84-0.87$, $P = 0.05$ and B = plants treated with SA by root application; correlation coefficient $r = 0.95$, $P = 0.05$. Symbols represent ▲ = shoot length and ● = root length.

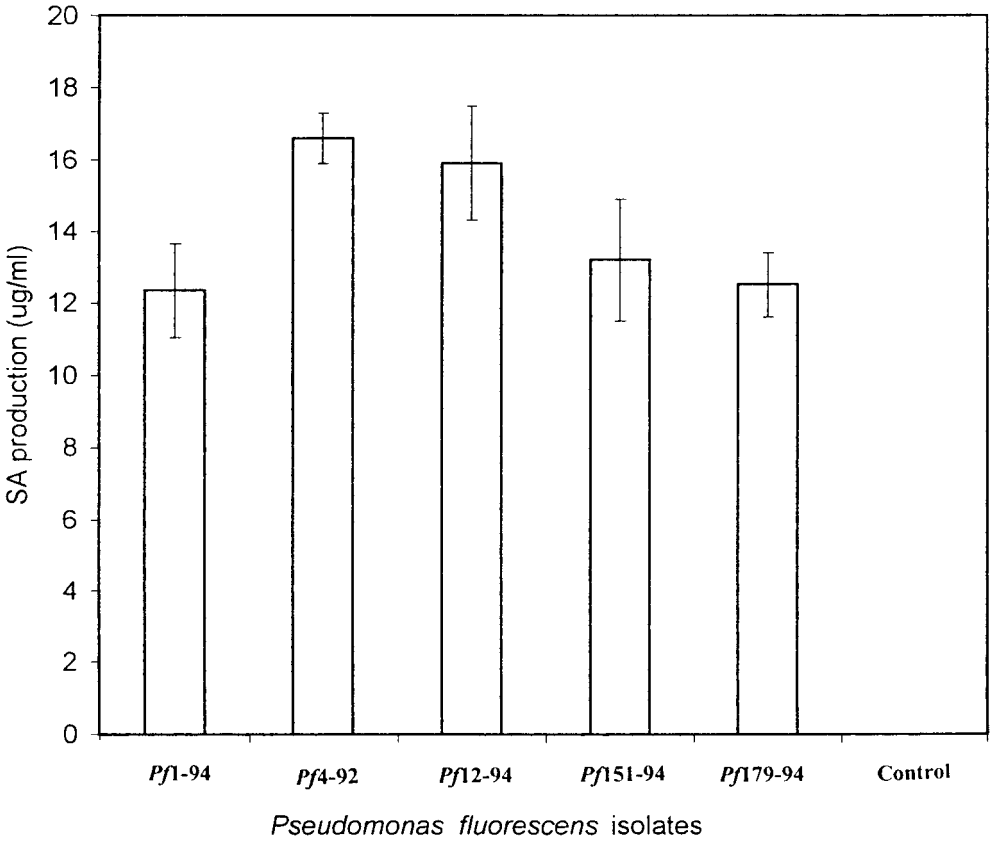

Fig. 5. Salicylic acid produced by isolates of *Pseudomonas fluorescens* | = S.D.

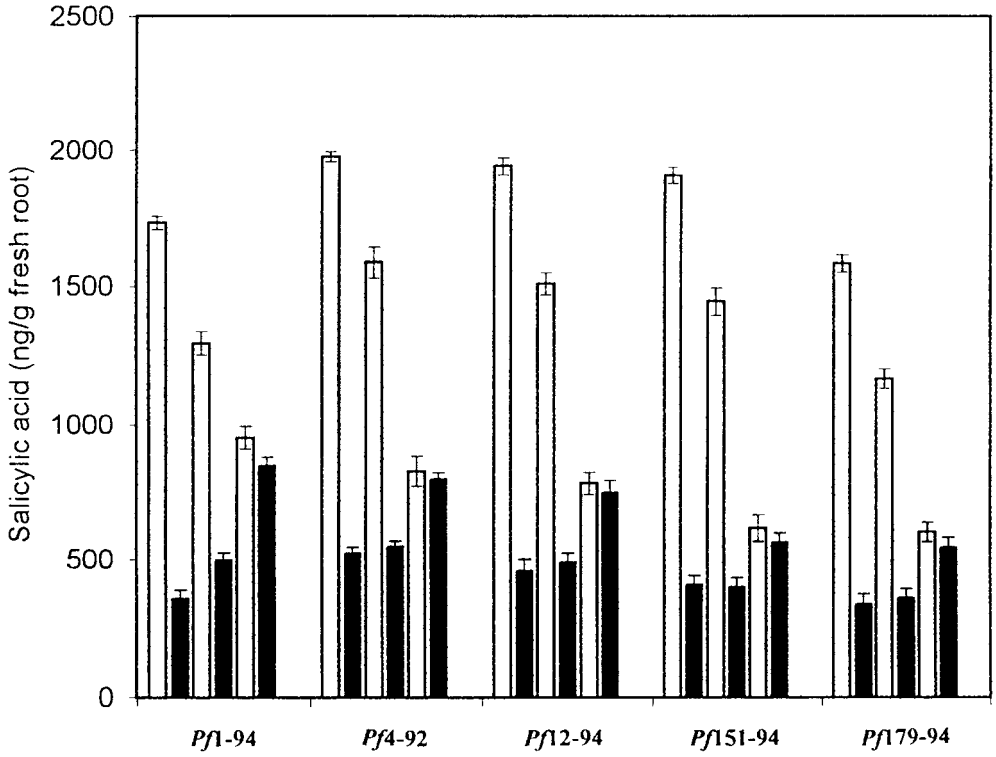

Fig. 6. Level of Salicylic acid in the roots of chickpea bacterized by *Pseudomonas fluorescens* isolates. Blank bars represent the level at site of application and filled bars represent the level at distant site at 1, 2 and 3 days after bacterization, respectively. | = S.D.

Discussion

Pseudomonas fluorescens have been widely used for plant growth promotion as well as for the management of plant diseases (Kloepper 1992; Meena *et al*. 2000; Kwack *et al*. 2002). The results demonstrate that *P. fluorescens* isolates increased the growth and controlled the severity of wilt disease of chickpea plant. Plant growth promotion by *P. fluorescens* is studied in detail and several mechanisms such as phosphate solublization, production of siderophore and plant hormones, greater rhizosphere competence etc. has been suggested for their growth promotion activity (Glick 1995; Van Loon *et al*. 1998). Induced physiological changes by application of the fluorescent pseudomonads have been demonstrated in several plants, which reduced the disease symptoms of a wide range of pathogens (Wei *et al*. 1991; Leeman *et al*. 1995; Liu *et al*. 1995; Hoffland *et al*. 1996). This phenomenon of induction of disease resistance may be dependent on salicylic acid accumulation or Jasmonic acid pathway (Van Loon *et al*. 1998). In this study, the effectiveness of *P. fluorescens* isolates through induced resistance against wilt of chickpea was evaluated. Significant level of disease control was observed by the isolates of *P. fluorescens*, which is significantly increased by combine effect of synthetic chemical resistance inducers with *P. fluorescens* isolates, whereas either of the treatments alone could not reduce the disease to that extent (Fig. 2). These results are in agreement with the work of Buchenauer and Vogt (1997). They observed that *P. fluorescens* + DL-B-aminobutyric acid (BABA) was more effective than BABA alone to control the damping off disease of cucumber caused by *Pythium ultimum*. Kataria *et al*. (1997) also reported that application of *P. fluorescens* with 5-nitrosalicylicacid, o-acetylsalicylicacid, 2, 6-dichloroisonicotinic acid, 2-aminoisobutyric and lichenan acid reduces the root rot caused by *Rhizoctonia solani* in bean and cucumber. They observed that combine application of *P. fluorescens* and chemical inducers were more effective than their individual application against this pathogen.

The result indicates that disease severity on the split root was significantly lower than the control (Table 1). Apparently, it confirms the triggering of plant mediated resistance mechanism i.e. induced systemic resistance by application of *P. fluorescens*. *Pseudomonas* mediated induced resistance has also been

demonstrated by other workers (Maurhofer *et al*. 1994; Liu *et al*. 1995; Park and Kloepper 2000). Though Van Loon (2000) discussed that rhizobacterially mediated induced systemic resistance does not require SA and is dependent upon ethylene and Jasmonic acid, many other workers have showed role of SA in ISR (Meyer and Hofte 1997; Chen *et al*. 1999; Audenaert *et al*. 2002). In this study also increased level of SA in roots indicate the metabolism of phenol in SA and is the first evidence about its role as resistance signal (Malamy *et al*. 1990; Rasmussen *et al*. 1991). In Petri-plate assay SA inhibited mycelial growth of *F. o. ciceri* at high concentrations i.e. 200–2000 µg ml^{-1} (Fig. 2). The result is in agreement with Chen *et al*. (1999) who also reported that SA inhibited the mycelial growth of *P. aphanidermatum* only at concentrations of 300–3000 µg ml^{-1}.

Exogenous SA stimulated the systemic resistance against *Fusarium* wilt of chickpea and reduced the disease severity significantly ($P = 0.05$; Table 2). The findings are corroboratory to White (1979) and Rasmussen *et al*. (1991), who observed that exogenous SA injection caused resistance against further infection by *Colletotrichum lagenarium* on cucumber leaves. Ability of exogenously supplied SA to induce resistance and expression of associated genes has already been demonstrated (Raskin 1992). Increased level of SA in bacterized roots was observed at the site of application as well as in distant roots (Fig. 6). However, at first day SA levels were significantly higher at bacterized site compared to distant site, which became almost equal at third day. The results suggest rapid biosynthesis of SA at the site of rhizobacterial colonization and then its translocation to other parts of root. Meuwly and Metraux (1993) also observed that free SA increased 33 – fold locally in the first infected cucumber leaf, and 4.2 – fold systematically in the second leaf compared to controls (5 days after infection by *P. lachrymans*).

Chen *et al*. (1999) reported that production of SA from *P. fluorescens* can not be correlated with its ISR activity. However, our observation indicates that *P. fluorescens* could stimulate chickpea plants to accumulate SA in their roots. It is not clear from our study that SA detected in bacterized chickpea root was produced by plant itself or by bacteria, whether the increased amount of SA is locally generated or systemically or transferred from bacterized or other tissues because free SA is translocated through plant phloem (Metraux *et al*. 1990; Rasmussen *et al*. 1991; Yalpani *et al*. 1991). Overall, our results demonstrate that *P. fluorescens* isolates can reduce the *Fusarium* wilt of chickpea. The combined application of *P. fluorescens* + chemical resistance inducers was more effective in reducing the *Fusarium* wilt. *P. fluorescens* can produce SA in culture as well as in induced root tissues of the chickpea. This study also demonstrates that both exogenous and biosynthesized SA can induce systemic resistance to *Fusarium* wilt of chickpea.

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