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Pseudomonas aeruginosa-mediated Induction of Systemic Resistance in Tomato Against Root-knot Nematode

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Abstract: Plant growth-promoting rhizobacterium *Pseudomonas aeruginosa* strain IE-6S$^+$ suppresses root-knot nematode (*Meloidogyne* spp) indirectly by enhancing defense mechanism leading to induced systemic resistance in tomato. However, which determinants are important in the induction of resistant reaction in plants against nematode by IE-6S$^+$ is yet fully understood. Salicylic Acid (SA) production by bacteria acts as endogenous signal for the activation of certain plant defense responses. In a split root trial with tomato plant as a host and *M. javanica* as challenging pathogen, IE-6S$^+$ induced systemic resistance in both wild type and NahG tomato seedlings. Moreover, the bacterial efficacy against nematode was not altered when soil chemical compositions was changed by the addition of iron. These results suggest that *P. aeruginosa* IE-6S$^+$ suppress root-knot nematode indirectly via enhanced defense mechanism in plants, which is independent of SA accumulation in the host.

Key words: Fluorescent pseudomonads, induced systemic resistance, secondary metabolites, root-knot nematodes, GM plants

INTRODUCTION

Some fluorescent pseudomonads are referred to as Plant Growth-promoting Rhizobacteria (PGPR) and their effectiveness in controlling a number of plant diseases caused by soil-borne pathogens has been widely documented[1,2]. In spite of the interest devoted to understanding the protective role of PGPR, deciphering the mechanisms by which these bacteria exert their activity has remained a challenge. For instance, competition for substrate and niche exclusion[3], antibiosis [4,5] or production of extracellular enzymes[6] has been described as mechanisms involved in disease suppression. Our recent studies have suggested that Induced Systemic Resistance (ISR) may play an important role in the suppression of *Meloidigyne javanica*, the root-knot nematode in tomato by *P. aeruginosa* strain IE-6S$^+$ [7-9].

The use of bacteria-mediated ISR for the control of plant-parasitic nematodes is still a new research area, and studies on the mode-of-action have only just started to be explored. For instance, Reitz *et al.*[10] identified purified LPS as the causal mechanism of systemic resistance induced by an endophytic bacterium *Rhizobium etli* G12 against the potato cyst nematode *Globodera pallida* in potato. Reitz *et al.*[10] further demonstrated that the resistant reaction triggered by *R. etli* G12 was not accompanied by enhanced accumulation of Pathogenesis-related (PR) proteins such as chitinase and β-1,3-glucanase. In a recent study, Siddiqui and Shaukat[9] found that secondary metabolite 2,4-diacetylphloroglucnol producing *P. fluorescens* strain CHA0 induced systemic resistance against root-knot nematode in tomato.

Salicylic Acid (SA) is known to play a critical signaling role in the activation of plant defense responses after pathogen attack[11]. SA sprayed on cowpea inoculated with *M. incognita* reduced nematode infection and induced expression and accumulation of pathogenesis related proteins in the leaves of sprayed plants[12]. Therefore, SA production by bacteria in rhizosphere may enhance defense mechanisms in plants leading to systemic resistance against a variety of soil-borne plant pathogens including plant-parasitic nematodes. In the recent study, *P. aeruginosa* strain IE-6S$^+$ was found to synthesize SA *in vitro* and elicited resistance reaction in tomato plants against root-knot nematode to a similar degree as synthetic SA in both iron-amended and non-amended soils[8]. However, in the absence of appropriate control i.e., SA-negative mutant of the strain IE-6S$^+$ and iron independent antagonism by bacterium against nematode, these authors concluded that SA was not involved in the induction of systemic resistance in tomato. In this study, in a split-root assay,

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we investigated the role of SA in the enhancement of systemic resistance in both wild type and transgenic (NahG) tomato plants using *M. javanica* as a challenging pathogen. The NahG gene encodes salicylate hydroxylase, which converts SA into catechol, a product that does not induce resistance. As the available iron in the soil affects rhizobacteria-mediated ISR, the ability of IE-6S$^+$ was determined in both iron deficient and iron sufficient soils.

MATERIALS AND METHODS

Organisms and culture conditions: The maintenance and culture conditions for *P. aeruginosa* strain IE-6S$^+$ have already been described[7]. Isolation and maintenance of root-knot nematode, *M. javanica* has described somewhere[8].

Induced systemic resistance: Both wild type (Cv. SUN (6002) PVP) and NahG tomato seedlings were used in this experiment to ascertain whether SA act as an inducing agent in the induction of systemic resistance against nematode in tomato. Three-week-old tomato seedlings were uprooted from sterilized soil, washed with tap water, and roots split into two halves with a sterilized dissecting scalpel. Each half of the root system was transplanted into separate 8 cm diam. plastic pots containing 350 g soil/pot attached together on the outside with a masking tape. A 10 cm long and 2 mm diam steel rod placed between the two pots provided support to the plant. One of the root system was treated with a 35 mL cell suspension (diluted to 10^8 cfu mL^{-1}) of *P. aeruginosa* strain IE-6S$^+$ prepared in sterile ¼ strength Ringer solution. Soil treated with 35 mL sterile ¼ strength Ringer solution served as controls. One week after seedling establishment, the other (untreated) half of the root system was infested with 2000 freshly hatched juveniles of *M. javanica*. The experiment was a Randomized Complete Block Design with 7 treatments (including a control) and 8 replications, each with one plant. Nematode invasion was assessed 21 days after inoculation from the nematode treated half following the procedure described by Siddiqui and Shaukat[7]. The experiment was performed three times.

Reisolation of *P. aeruginosa* from rhizosphere: To determine whether bacteria applied to one half of the split root system migrate systemically to the other half of the root system and contribute in the induction of systemic resistance against nematode, in a separate experiment, the bacteria were isolated from the rhizosphere of the non-bacterized half of the root system by the method described earlier[7].

Effect of soil iron status on biocontrol of *M. javanica*: Three-week-old wild type tomato seedlings were planted in plastic pots (8 cm diam) filled with 300 g unsterilized sandy-loam (pH 8.1; moisture retaining capacity 38%), and cultivated in a glasshouse (19-24 and 29-33°C day and night temperatures, respectively). After one week, the plants were treated with bacteria by pipetting 30 mL of the bacterial suspension ($3.3x10^8$ cfu mL^{-1}, prepared in ¼ concentration Ringer solution) into soil around the root system. Control plants received 30 mL of ¼ concentration Ringer solution. Two days after bacterial application, 2000 freshly hatched juveniles of *M. javanica* were added to the soil by making three holes around the seedlings. To demonstrate if lowered iron availability in soil influenced biological control activity of the bacteria, in another experiment, in addition to the bacterial treatments described above, the tomato seedlings were treated biweekly with 15 mL of 10 μM of an iron chelator, ethylenediamine di(o-hydroxyphenylacetic acid) (EDDHA) solution as a soil drench[13].

The root samples were taken 45 days after nematode inoculation. Tomato roots were carefully rinsed in tap water, separated from the shoot, blotted dry and weighed. The numbers of galls produced on the entire root system were counted using a hand lens. Final population densities of the nematode in the roots were estimated following the method outlined in Siddiqui and Shaukat[7].

Statistical analysis: Data were subjected to analysis of variance (ANOVA) or factorial analysis of variance (FANOVA), followed by Least Significant Difference (LSD) test at p=0.05 and Duncan's Multiple Range Test to compare treatment means, using STATISTICA ver.5.0 software. Where variances between repeated experiments were similar, the analysis was performed on the pooled data.

RESULTS AND DISCUSSION

In two of the three split root trials, application of *P. aeruginosa* strain IE-6S$^+$ to one half of the split root section reduced nematode final population densities to other (non-bacterized nematode treated) half of the root system in both wild type and NahG tomato (Fig. 1). However, in one the trial presented, strain IE-6S$^+$ reduced nematode populations in wild type but not in transformed NahG tomato seedlings. Irrespective of the treatment, nematode populations were markedly higher in genetically modified NahG tomato seedlings compared to the wild type plants. Strain IE-6S$^+$ applied to one half of the root system was not isolated from the non-bacterized nematode-treated half of the split root

Table 1: The influence of soil with *Pseudomonas aeruginosa* on root-knot development by *Meloidogyne javanica* nematode final population densities and fresh root weight of tomato plants

	Galls/root system		Juveniles/root System		Fresh root weight (g)	
Bacterial strains	EDDHA$^-$	EDDHA$^+$	EDDHA$^-$	EDDHA$^+$	EDDHA$^-$	EDDHA$^+$
None	142a	159a	234a	255a	2.1a	2.3a
P. aeruginosa	97b	123b	189b	206b	2.4a	1.9b
$LSD_{0.05}$	35	31	40	45	0.5	0.7

EDDHA = ethylenediamine di(o-hydroxyphenylacetic acid); LSD = least significant difference; Values in the same column with different letter are significantly different at $p<0.05$ in accordance with Duncan's multiple range test

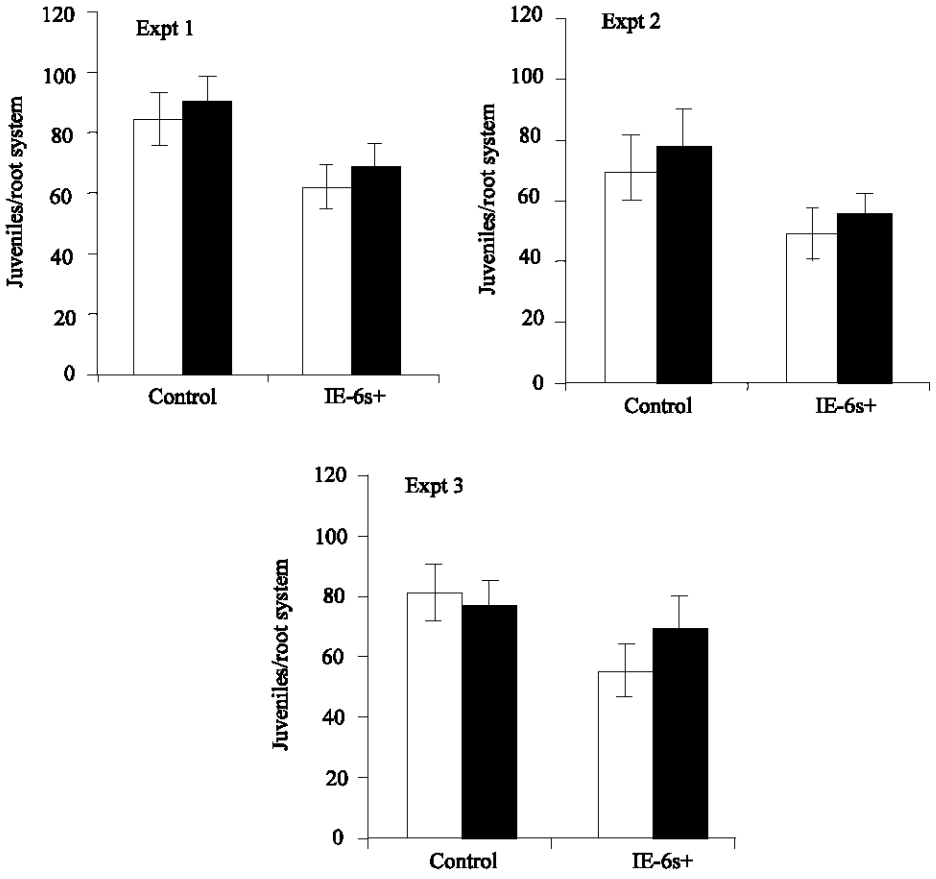

Fig. 1: Number of juveniles of *Meloidogyne javanica* which had penetrated the root of the untreated side of a split root system in both wild type □ and transgenic (NahG) ■ tomato plants. The other side was inoculated with *Pseudomonas aeruginosa* strain IE-6S$^+$. Each bar represents one standard deviation. Since error variance between repeated experiments was significantly different therefore results of the individual experiments are presented separately.

system (data not presented). These results clearly indicate that *P. aeruginosa* strain IE-6S$^+$ induces systemic resistance in tomato, which is independent of SA biosynthesis in host roots. From a number of studies, it is now quite evident that SA production by rhizobacteria is involved in the induction of systemic resistance against specific soilborne pathogens. Maurhofer *et al.*[14] showed that *P. fluorescens* strain P3 engineered to produce SA had an improved capacity to induced systemic resistance against tobacco narcotic virus in tobacco. Similarly, De Meyer and Höfte[15] observed that SA-negative mutants of *P. aeruginosa* 7NSK2 lost their ability to induce systemic resistance against *Botrytis cinerea* in bean. However, similar to our current study, Press *et al.*[16] suggested that SA production by *Serratia marcescens* strain 90-166 was not important in the induction of systemic resistance in cucumber and tomato, because SA-negative mutants of strain 90-166 induced the same level of resistance in

cucumber, wild type tobacco and NahG-tobacco (the NahG gene encodes the enzyme salicylate hyroxylate that degrades SA) expressing salicylate hydroxylate as the wild type. More recently, Siddiqui and Shaukat[17] using SA-negative mutants of *P. aeruginosa* 7NSK2 or SA overproducing derivative of *P. fluorescens* strain CHA0 found that insertion or deletion of phlA genes (which encodes the production of SA in bacteria) neither reduced nor optimized the bacterial capacity to induce systemic resistance against *M. javanica* in tomato. Mutants induced same level of resistance against *M. javanica* in both wild type and NahG tomato plants. A role of SA in rhizobacteria-mediated ISR seems to depend on the biocontrol strain, plant species and plant pathogen.

Effect of soil iron status on biocontrol of *M. javanica*: When compared to the control, soil application with *P. aeruginosa* strain IE-6S$^+$ significantly ($p<0.05$) suppressed root-knot nematode galling and nematode final population densities in both iron deficient (soil iron status was lowered by he addition of iron chelator, EDDHA) and iron sufficient soil (un-amended) soils (Table 1). Regardless of the bacterial treatment, galling induced by *M. javanica* and nematode final population densities were markedly higher in iron deficient soil compared to the iron sufficient soil. Whereas fresh root weight remained unaffected following introduction of the bacterial inoculant in iron sufficient soil, IE-6S$^+$ significantly reduced root weights in iron deficient soils compared to the controls. Rhizobacteria-mediated ISR may be affected by iron availability. For instance, the ability of *S. marcescens* 90-166 to induce resistance in cucumber decreased with increasing iron concentration in the fertilizer[16]. Some *Pseudomonas* strains reduced Fusarium wilt of radish more effectively when iron availability in the nutrient solution was low[18]. *P. aerugnosa* 7NSK2 triggered ISR against *B. cinerea* in bean when the bacterium was cultivated on iron-poor, but not ion iron-rich, media[15]. These findings suggest that bacterial sideropores, which in general are produced under conditions of iron limitation, might involved in ISR against soilborne fungi and viruses. However, the results of the current as well as our previous studies[17,19] clearly indicate that rhizobacteria suppresses root-knot nematode infection, which is not affected by the soil iron availability.

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