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INDUCTION OF DISEASE RESISTANCE IN COMMON BEAN SUSCEPTIBLE TO HALO BLIGHT BACTERIAL PATHOGEN AFTER SEED BACTERIZATION WITH RHIZOSPHERE PSEUDOMONADS

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With the aim of elucidating the immunizing ability of plant-growth-affecting rhizosphere pseudomonads, seeds of a susceptible bean (*Phaseolus vulgaris* L) cv. Bonita, were subjected to bacterization before challenging with the halo blight bacterial pathogen *Pseudomonas syringae* pv. *phaseolicola*. In the greenhouse, induced systemic resistance to halo blight was found in bean plants treated with a plant growth stimulatory strain of *P. fluorescens* (S 97), whereas deleterious pseudomonads MA 250 and VS 50 were found to induce susceptibility towards the disease. Immunization ability of S 97 was reduced at low inoculum densities ($< 10^7$ live cells per ml) or eliminated when the suspension was autoclaved. The maximum disease protection, measured in terms of number of halo blight lesions in trifoliate leaves, was obtained at the highest inoculum concentration (10^8 live cells per ml). Agar diffusion assay in vitro revealed that S 97 exhibits bacteriostatic activity against the bean pathogen. It is suggested that S 97 might evolve substances already during seed germination that are translocated to the foliage; there they might accumulate around the site of bacterial multiplication and contribute to their restricted growth.

Halo blight of beans (*Phaseolus vulgaris* L) is caused by *Pseudomonas syringae* pv. *phaseolicola* (Young, Dye, and Wilkie 1978). The pathogen enters plants through wounds or stomata during periods with high relative humidity or free moisture. The use of disease-free seeds is generally considered to be the only effective method of controlling *P. s.* pv. *phaseolicola.*

Kuc' (*10*) has proposed that all plants have a latent genetic potential for resistance mechanisms to bacterial as well as to fungal and viral diseases. Both

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abiotic and biotic agents have been used to provide protection against pathogens in plants (*10, 13*). A classical example is *Colletotrichum lindemuthianum*, which infects beans. Susceptible cultivars of beans have successfully been protected against this disease by exposing them to nonpathogenic or pathogenic races of this pathogen and to *C. lagenarium* (*8, 9*).

Other microbial agents that are assumed to have the potential to elicite disease resistance are plantgrowth-promoting rhizobacteria (PGPR) (*4*). Inoculation of crop plants with PGPR has in a large number of investigations resulted in increased plant growth and yield both in the greenhouse and in the field (*14*). The beneficial effects of these bacteria have in most cases been related to their ability to produce plant growth hormones and/or antimicrobial substances and to protect growing roots from deleterious root microorganisms present in the rhizosphere (*3, 11, 14, 15*). However, the beneficial effects have not always been convincingly explained by these mechanisms. The importance of PGPR in disease resistance has been the subject of few investigations, yet such rhizobacteria might contribute to increased plant dry matter due to induced resistance towards various diseases in plants.

The fact that we and several other investigators have obtained plant growth promotion after inoculation with PGPR without relating this effect directly to above-mentioned modes of action has initiated this study (*2, 12*). This paper reports about systemically induced resistance/susceptibility to halo bacterial blight in a susceptible bean cultivar. The resistance is induced by a rhizosphere pseudomonad with plantgrowth-promoting ability. The objective of this study was also to test the simple method of seed bacterization for plant immunization, which is more suitable for routine use than stem or leaf inoculation (*7*).

MATERIAL AND METHODS

Bacterial strains and culture media. *Pseudomonas syringae* pv. *phaseolicola*, Psp 52, race 1 (from NCPPB) pathogenic to common beans was used as the challenger strain. The inducer strains S 97 (described in 2), MA 250, and VS 50 have earlier been isolated from plant rhizospheres and identified according to API identification system 20 NE as *Pseudomonas fluorescens* (S 97, MA 250) and *P. maltophila* (VS 50). The first two strains were chosen for their ability to suppress several fungal pathogens in vitro. S 97 was found to stimulate growth of lettuce and beans in longer lasting greenhouse experiments (*2*), and of potatoes in field studies in Sweden (Hökeberg, data unpublished). VS 50 is a nonpathogenic saprophyte. All the strains were grown from frozen or lyophilized cultures on King's medium B agar (KBA, *6*) for two days and then suspended in 0.01 M $MgSO_4$ solution. The concentration of bacterial suspensions were adjusted to approx. 10^8 cfu/ml.

Plant material and seed bacterization. Bean seeds of the susceptible *P. vulgaris* cultivar Bonita were shaken for two hours at room temperature in the bacterial suspensions made from inducer strains and for controls in 0.01 M $MgSO_4$. The seeds were then sown in eight pots containing moist greenhouse peat soil (*5*), 2 per

pot (10 cm^3), and placed under a plastic cover in a greenhouse with a night and day temperature regime of 20°C/25°C respectively. Artificial light was supplied only on cloudy days with Phillips HPI-T mercury lamps, 400 W. Plants were watered daily and fertilized frequently with Superba S liquid fertilizer (6.5% N, 1.0% P, 4.7% K plus microelements, Supra Ceres, Landskrona, Sweden). Plant response as a result of seed bacterization prior to challenging was recorded in terms of emergence, plant length after one week, and shoot dry wt. after 3–4 weeks of sowing.

Procedure for challenging plants. The first trifoliate leaf of all emerged plants was challenged with the pathogenic strain Psp 52 by making a 1-cm cut in the top of all leaflets of the trifoliate leaf and dipping the cut portions in the bacterial suspension. Plants were covered with plastic bags for 3 days in order to retain moisture favorable for disease development. Lesions due to halo blight were counted in the third trifoliate leaf after 10–15 days and shoot dry weight was recorded at the end of an experimental period of about six weeks.

Effect of bacterial concentration on induced resistance. This experiment was carried out with the rhizobacterium S 97, which gave promising results in initial experiments. A fresh suspension of S 97 in 0.01 M $MgSO_4$ solution (4.6×10^8 cfu/ml) was diluted down to 4.6×10^4 cfu/ml. The bean seeds were shaken in the various dilutions as described above. One control with 0.01 M $MgSO_4$ solution and another with autoclaved bacterial suspension with the highest concentration were also included. The treated seeds were sown in 8 pots/treatment (2 seed per pot) as mentioned above and placed in a greenhouse with similar growth conditions. Emergence and plant length were recorded before challenging the plants with Psp 52 according to the above procedure. Halo blight lesions were counted 10–15 days after challenging, and shoot dry weight was recorded after drying them at 85°C overnight. All experiments were repeated twice and data analyzed statistically according to ANOVA.

In vitro antibiosis by P. fluorescens, S 97 against Psp 52. Three different media, KBA, NA (nutrient agar 28 g/*l*), and TSA (10 g/*l* tryptic soy broth and 12 g/*l* technical agar) were tested for detecting the antibacterial activity of S 97. A fresh suspension of Psp 52 was spread evenly on two plates of each agar. After 3 hours, S 97 suspension was put into four holes of equal size punched into the agar. Control agar plates were prepared in a similar way except that the holes were filled with sterile 0.01 M $MgSO_4$ solution, and the inhibition zone, if any, was recorded after a 72-h incubation at room temperature.

RESULTS

Plant reaction to rhizosphere pseudomonads

As a result of seed bacterization, bean emergence was reduced to some extent by all three tested *Pseudomonas* strains (Table 1). The plant response due to S 97 at the end of the short experimental period, i.e. 3 weeks, was growth stimulatory, but the effect was not statistically significant. Further, MA 250 and VS 50 were

Table 1. Effect of seed bacterization with rhizosphere pseudomonads on germination and growth of bean cv. Bonita.

Strain	% Emergence	Average plant length (cm.)[b]	Average shoot dry wt. (g.)[b]
P. fluorescens, S 97	50	21.6	1.12
P. fluorescens, MA 250	38[a]	13.5[a]	0.64[a]
P. maltophila, VS 50	44[a]	17.4	0.76[a]
None (0.01 M $MgSO_4$)	63	19.1	1.04

[a] Within each column appear the mean values per plant that are significantly different from the controls at $p = 0.01$

[b] Three weeks after sowing.

Table 2. Immunizing ability of rhizophere pseudomonads in susceptible bean cv. Bonita against *P. syringae* pv. *phaseolicola*. 52.

Inducer isolate	Average no. of lesions in 3rd trifol. leaf/plant	% plants with symptoms in 3rd trifol. leaf
P. fluorescens, S 97	0.5ax	17a
P. fluorescens, MA 250	77b	100b
P. maltophila, VS 50	128b	100b
None (0.01 M $MgSO_4$)	33c	100b

x Means within same column with different letters are significantly different ($p = 0.01$).

found to significantly reduce emergence as well as to be deleterious to plant growth.

The slightly harmful effect of S 97 on bean emergence was found to be dose-dependent, as this effect, neither in terms of plant length nor shoot dry wt., could be measured at harvest when compared to the 0.01 M $MgSO_4$ controls. This effect was fully eliminated at the concentration 4.6×10^7 cfu/ml or when the initial inoculum was autoclaved (data not shown). Antibiosis test further showed that this strain inhibited growth of the pathogen Psp 52 only on one of three media tested. A 3-mm inhibition zone was developed on KBA around the holes filled with S 97.

Expression of induced resistance

No direct growth effects of treatment with either sterile 0.01 M $MgSO_4$ or autoclaved suspension of S 97 were observed in unchallenged control plants. Seed bacterization with live cells of S 97 resulted into a minimum number of halo blight lesions in the susceptible bean cultivar Bonita challenged with the pathogen Psp 52 (Table 2). There was a clear tendency for a dose response relationship as the number of lesions decreased with the increasing concentration of induction inoculum (Fig. 1). Less variation in lesions/leaf within a treatment was also observed when S 97 was applied at the highest concentration, compared to when it was diluted. Seed treatment with the autoclaved initial inoculum eliminated most, but not all, of the immunizing effect. However, no significant difference was found

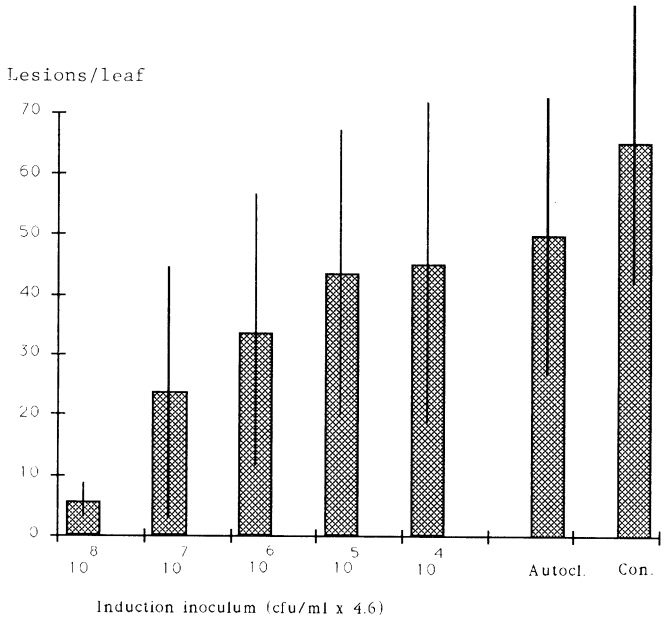

Fig. 1. Systemic induced resistance with *P. fluorescens*, S 97 to halo blight bacterial pathogen *P. syringae pv. phaseolicola*, 52, race 1 as a function of inoculum concentration at seed bacterization. The vertical bars show standard deviation.

between this treatment and the controls that were treated with 0.01 M $MgSO_4$ solution prior to challenging (Fig. 1).

In contrast, treatment with MA 250 and VS 50, which were shown to be harmful to plant growth (Table 1), resulted in a statistically significant increase in number of lesions in the emerged plants (Table 2) when compared to control plants, thus revealing their ability to promote susceptibility of bean plants towards the pathogen Psp 52.

DISCUSSION

These result demonstrate that a susceptible cultivar of bean can be immunized against the foliar pathogen Psp. 52 by using certain rhizosphere-inhabiting bacteria. Here, the resistance response was elicited in the plants when the inducer bacterium, S 97, was applied on seeds. Earlier studies on plant immunization have been made by applying the inducer agent on the surface or by infiltrating the leaf or stem, and immunization was thus achieved in plant parts above the inducer leaf (*1*, *10*). On the basis of results presented here, seed bacterization seems to offer a possibility of immunizing the whole plant against bacterial diseases.

Seed inoculation in greenhouse tests with two other rhizosphere-inhabiting *Pseudomonas* strains, MA 250 and VS 50, which have been shown to be plant

growth-deleterious, resulted in increased susceptibility towards the disease caused by halo blight pathogen. The inoculum load applied to seeds in these experiments was generally 10^8 cfu/ml, which may be high enough to cause significant growth inhibition in bean cv. Bonita. In earlier studies, this cultivar has been shown to be sensitive to treatment for many deleterious rhizosphere bacteria (*2*). The strain S 97 exerted no harmful growth effects on bean plants. But if the effect of MA 250 and VS 50 on plants and their susceptibility towards halo blight is dose-dependent is yet to be tested.

The prerequisite for maximum immunization was the highest viable cell concentration used, i.e. 4.6×10^8 cfu/ml of S 97, as the disease protection decreased with lower dilutions (Fig. 1). From this finding it can be concluded that the concentration of inoculum used for induction is important for the extent of resistance induced in the plant. This is in agreement with Kuc' (*10*), who also emphasized that the concentration of inoculum used for induction and the number of lesions produced on the inducer leaf are directly related to the extent of immunization until a saturation point of inoculum or lesions is attained. S 97 at the highest concentration, however, suppressed the bean seed germination slightly. When applied at 4.6×10^7 cfu/ml, seed germination was normal and the bean plants were still protected significantly against the halo blight pathogen. These observations suggest that the critical level of inoculum required for optimum protection and at the same time elimination of the slightly negative effect on seed emergenece may lie between 4.6×10^8 cfu/ml and 4.6×10^7 cfu/ml. In the present study using the seed as the induction site, the number of live cells of S 97 colonizing per seed were not counted, and hence the minimum load of viable cells per seed essential for attaining maximum emergence and disease protection is yet to be investigated.

Kuc' (*10*) found that both the fungal components and its metabolites could immunize the plants against fungal pathogens. Bacterial strain S 97 used here is a PGPR according to several longer lasting experiments performed in our laboratory (*2*). It is also known to exude nonspecific fungal toxins (*2*). In this study, agar diffusion tests have further revealed that this strain restricts the growth of Psp 52 only on KBA medium, which means that it exudes substances antagonistic towards the pathogen under low iron conditions. In a greenhouse experiment, the culture filtrate of S 97 was to some extent also found to immunize the bean plants against the pathogen. Since more work is required to confirm this finding, the conclusion can not be drawn that the substances involved in immunization are the same as the bacteriostatic substances produced by the strain S 97.

If the immunization is only against the expression of symptoms or also against bacterial multiplication is yet to be explored. Nevertheless, the strong reduction in the number of halo blight lesions in the presence of living inoculum and the elimination of this ability after autoclaving of the suspension seem to suggest that S 97 might evolve substances already during seed germination, which are then translocated to the foliage and there might accumulate around the site of bacterial multiplication and contribute to the restricted growth of the bacteria.

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