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Susceptibility of Various Tomato and Lettuce Genotypes to Plant-Growth-Promoting *Pseudomonas**

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Abstract

The plant rhizosphere is frequently colonized by fluorescent pseudomonads. *Pseudomonas fluorescens-putida* cluster in the bosom of rhizosphere and rhizoplan can be prevailing for some plant genotypes. In this study, the responses of several genotypes of lettuce (*Lactuca sativa* L.) and tomato (*Lycopersicon esculentum* Mill.) to inoculation under controlled conditions with one *P. fluorescens* selected strain were analysed. The obtained results concerned cotyledons surface and fresh weight for lettuce, emergence and cotyledons and first true leaf length for tomato. They allow to assess precisely plant-growth promotion and to demonstrate that certain genotypes are more susceptible than others to plant-growth stimulation effect caused by these bacteria. Disparity of susceptibility between these genotypes suggest a relative microbial dependance of certain plant genotypes with respect to the growth promoting effect.

1. Introduction

When ecological conditions are favourable, lettuce and tomato plant rhizospheres harbour a microflora where fluorescent *Pseudomonas* can frequently be prevailing colonizers from the first stages of the plant growth.

Some strains of *Pseudomonas fluorescens-putida* group isolated from lettuce and tomato rhizospheres exhibit an *in vitro* antagonism to potential invaders such as certain soil-borne fungi. Several studies point out a direct correlation between *in vitro* characters of *Pseudomonas* strains – specially antagonism – and their capacity to increase plant growth (Kloepper and Schroth, 1981, Digat and Gardan, 1987). But although growth promotion can result from biological control of classic plant diseases (Baker and Defago, 1987) i.e. from "indirect promotion" effect mainly due to a displacement of resident or deleterious microflora, it was recently demonstrated a

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growth promotion effect caused by *Pseudomonas* strains in the absense of disease symptoms (Digat et al., 1984, Kloepper et al., 1988).

The influence of plant genotypes on the rhizosphere population in general has been demonstrated (Neal et al., 1970). But there are few reports concerning the variability of interactions between P.G.P.R. (Plant-Growth Promoting Rhizobacteria) and plant genotypes.

The purpose of this study was to point out the variability of susceptibility at certain critical stages of several lettuce and tomato genotypes inoculated by one selected bacterial strain of *Pseudomonas fluorescens*.

2. Material and Methods

Plant material

Eight genotypes of lettuce (*Lactuca sativa* L.) and three genotypes of tomato (*Lycopersicon esculentum* Mill.) were used in this investigation. (Table 1).

Table 1. Codes and growing types of lettuce and tomato genotypes

Plant	Genotype code	Growing type
lettuce	L1, L3	Greenhouse "Batavia" (two lots A and B)
	L5, L6, L7	Greenhouse and field
	L8, L9, L10	"butter head"
tomato	T5	Field F1 hybrid
	T6	Greenhouse and field F1 hybrid
	T10	Greenhouse F1 hybrid

Plant cultivation in substrates

Plants were grown in controlled conditions: temperature 20°C, relative humidity 85%, light intensity of 10 000 lux with a photoperiod of 12 hours day/12 hours night. The cultivation was performed in plastic containers of $45 \times 30 \times 7$ cm with 200 plants for each treatment in fresh mould made of a mixture of brown peat and black peat (1:1). A nutrient solution at pH 5.8 was used for irrigating. The treatments were replicated six times.

Bacterial strain

The L26.1 bacterial strain was isolated from a lettuce rhizosphere in a sandy loam soil (pH 5.9) at Brain-sur-l'Authion (France). The strain was characterized as belonging to the *P. fluorescens* species according to the classic determinative scheme (Stanier et al., 1966). It was selected because its capacity of *in vitro* antagonism against a broad host range of pathogens (Digat and Gardan, 1987).

Plant bacterization, dynamic of inoculated bacterial population and root colonization

The bacterial strain was grown in a shaken (160 R.P.M.) medium (Misaghi et al., 1983). The stationary phase of 10^9 c.f.u./ml was attained after 48 hours at 25°C. Bacterial concentration was adjusted by turbidity (turbidimeter Hach 43 900). To obtain a theoretical concentration of about 10^6 c.f.u. and 10^4 c.f.u. per gram of substrate, 100 ml of the bacterial suspension at 10^7 c.f.u./ml and 10^5 c.f.u./ml respectively were carefully mixed to 1000 grams of substrate prior to sowing. An unbacterized control was used for each lettuce and tomato genotype. Dynamics of the bacterial population on the roots and in the substrate were followed by taking samples every three days. By isolation, serial dilution and streaking on King' medium B (King et al., 1954) bacterial colonies were obtained and identified by the sero-agglutination technique using a specific antiserum made from a bacterial glycoprotein extract as antigen (Digat and Cambra, 1976).

Plant growth promoting effects

To assess precisely the plant growth promoting effects:

- on the 7th day after bacterization, the mean percentage of tomato seedlings emergence was determined. Cotyledons surfaces of twenty lettuce plants taken at random in each treatment and control were determined by measuring length and width,
- on the 11th day, cotyledons lengths of ten tomato plants taken at random in each treatment and control were measured,
- on the 18th day, lengths of the first true leaf of ten tomato plants taken at random in each treatment and control were measured,
- on the 22nd day, mean fresh weights of lettuce seedlings of seven genotypes were determined: 200 young plants were cut off and aerial parts were weighed for each treatment.

Statistical analysis

Results were analysed by analysis of variance followed by Duncan's test to check if the difference between the averages was significant or not.

3. Results

Bacterial colonization of roots and substrate

Ten days after bacterization, *P. fluorescens* strain L26.1 was recovered in mean number 1.7×10^5 c.f.u./g in the substrate and 10^3 c.f.u./cm on the lettuce and tomato roots whatever the inoculum starting dose was. No *P. fluorescens* L26.1 was recovered in the control.

Plant growth responses to bacterization with Pseudomonas fluorescens L26.1

On lettuce cotyledons stage (Table 2) the plant-growth stimulating effect caused by the bacterization is much higher for genotype L3 (average 41.1%) than for genotype L1 (average 19.3%). However the inoculum dose 10^4 or 10^6 c.f.u./g of substrate has no significant influence on plant growth disparity. Twenty-two days after bacterization (Table 3) lettuce fresh weight of genotype L5 gave no response and genotype L9 a very weak one. But genotype L8 had an increased fresh weight up to 36.5% versus nonbacterized plants.

Table 2. Lettuce cotyledons surfaces 7 days after bacterization with *Pseudomonas fluoresens* L 26.1. A: Surface averages. B: Surface increase % v.s. control.

A. Surface averages

	Genotypes			
	L1		L3	
Treatment	A	B	A	B
Control	0.856	0.810	0.745	0.717
10^4 c.f.u./g	1.021**	1.005**	0.995**	1.064**
10^6 c.f.u./g	0.965**	0.980**	1.040**	1.023*

Note: Values (cm2) with ** and * are respectively significantly different at P=0.01 and P=0.05 from the non bacterized plants by Duncan's test

B. Surfaces increase % v.s. control

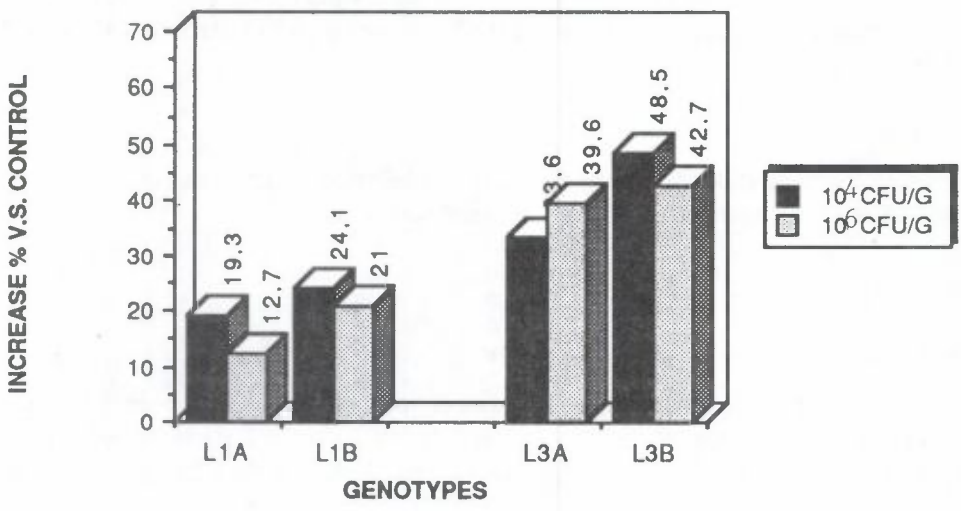


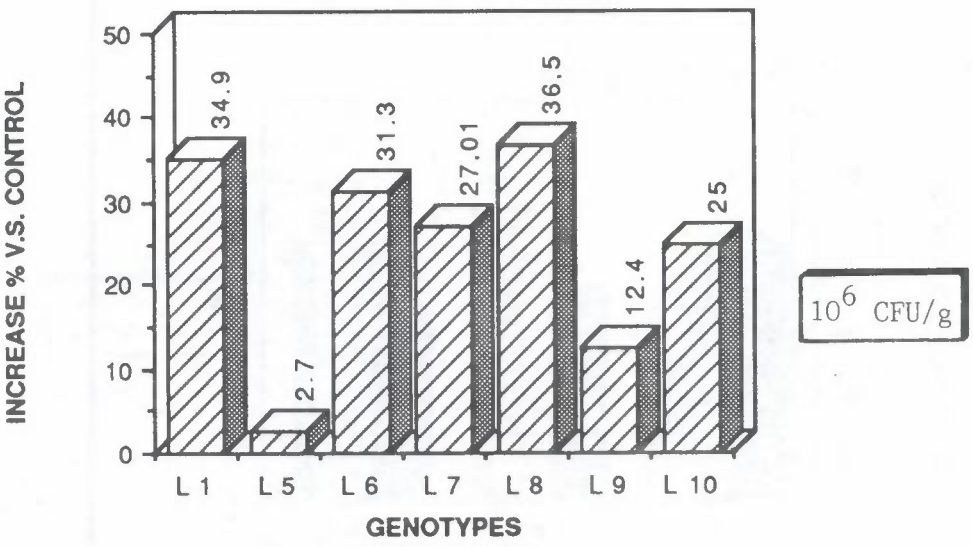
Table 3. Lettuce fresh weight 22 days after bacterization with *Pseudomonas fluorescenc* L26.I
A: Fresh weight averages. B: Fresh weight increase % v.s. control.

A. Fresh weight averages

	Genotype						
Treatment	L1	L5	L6	L7	L8	L9	L10
Control	1.583	1.785	1.485	2.544	1.784	1.965	2.633
Bacterized	2.135**	1.833	1.950**	3.245**	2.435**	2.209	3.291**

Notes: Values were determined for 10^6 c.f.u./g of substrate. Values for 10^4 c.f.u./g of substrate were not determined. Values (g) with ** are respectively significantly different at $P = 0.01$ from the non bacterized plants by Duncan's test

B. Fresh weight increase % v.s. control



For tomato genotypes T5, T6 and T10, emergence increase (Table 4) ranged v.s. control from +37.9% to 45.4% with a weak dose of inoculum (10^4 c.f.u./g). But response was rather homogeneous for these three genotypes. On cotyledons stage (Table 5), genotype T5 gave the best response 26.1% to the growth promoting effect and genotype T10 a weak one 12.9% even if it was bacterized with a high dose of inoculum. At first true leaf stage (Table 6) 18 days after bacterization, best responses were obtained with a weak dose of inoculum and the genotypes T5, T6, T10 showed an homogeneous susceptibility with a length increase percentage of 25.5, 29.7 and 25.8% respectively.

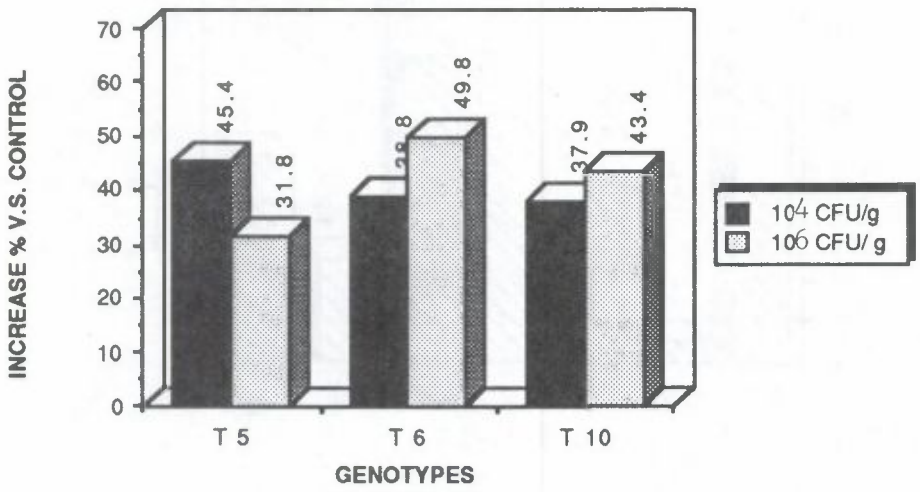
Table 4. Tomato seedlings emergence 7 days after bacterization with *Pseudomonas fluorescens* L26.1. A: Seedling emergence averages. B: Seedling emergence increase % v.s. control

A. Seedling emergence averages

	Genotype		
Treatment	T5	T6	T10
Control	33	22.7	27.2
10^4 c.f.u./g	48**	31.5 (NS)	37.5*
10^6 c.f.u./g	43.5**	34.0 (NS)	39.0*

Notes: Values are determined on lots of fifty seeds. Values with **, * and NS are respectively significantly different at P=0.01, different at P=0.05 and not significantly different from the non bacterized plants by Duncan's test

B. Seedlings emergence increase % v.s. control



4. Discussion

According to the genotype, bacterization of lettuce and tomato seedlings by *Pseudomonas fluorescens* L26.1 in controlled conditions resulted sometimes in increased plant growth compared to nonbacterized control. Twenty-two days after bacterization, lettuce genotype L8 increased as much as 36.4% v.s. control and 18 days after bacterization, first true leaf length increase of tomato genotype T6 was as large as 29.7% over the control. At this time, disparity of lettuce genotypes susceptibility to plant growth promoting effect was evident because genotypes L5 and L9 gave

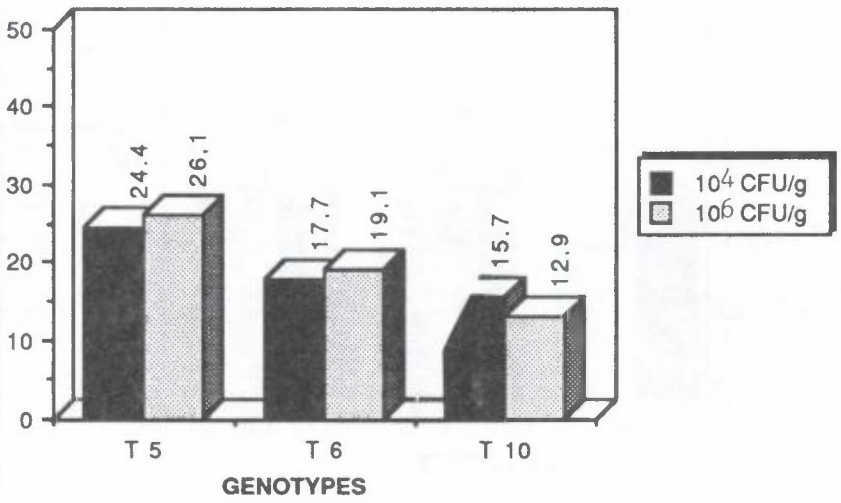
Table 5. Tomato cotyledons length 11 days after bacterization with *Pseudomonas fluorescens* L 26.1. A: Length averages. B: Length increase % v.s. control.

A. Length averages

	Genotype		
Treatment	T5	T6	T10
Control	2.34	2.20	2.71 [4]
10^4 c.f.u./g	2.91**	2.59**	3.14**
10^6 c.f.u./g	2.95**	2.62**	3.06**

Notes: Values (cm) with ** are significantly different at P=0.01 from the non bacterized plants by Duncan's test

B. length increase % v.s. control



practically no response while others such as L1, L6 and L8 were very stimulated. On the contrary, tomato genotype responses were more homogeneous and, differences in susceptibility between the genotypes observed at 7 and 11 days after bacterization were reduced, 18 days after bacterization.

The data reported here suggest that the plant-growth promoting effect caused by PGRG could vary not only with the plant genotype but also according to the growth stage. For example, a strong effect was observed on the emergence stage (Table 4) of the tomato genotype T5, but 11 days later on the true leaf stage the others genotypes had recovered their delay (Table 6).

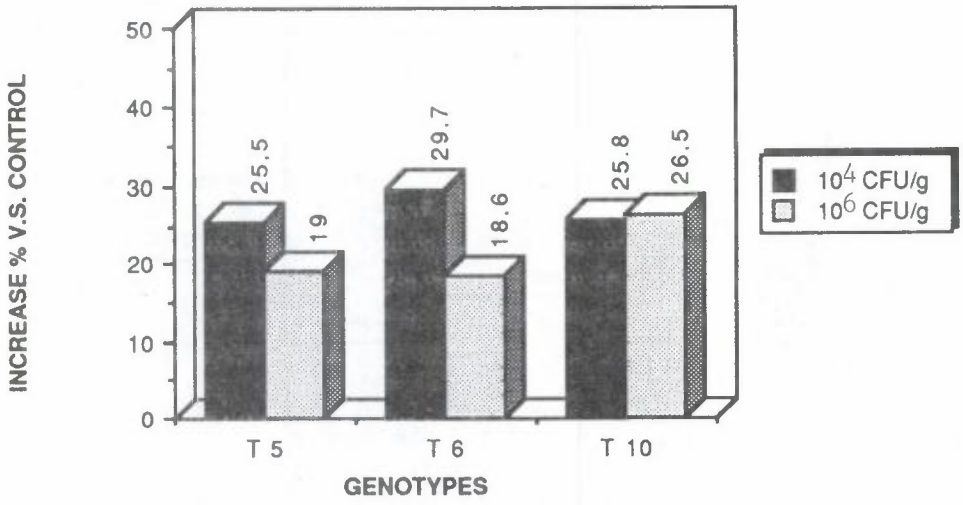
Table 6. Tomato first true leaf length 18 days after bacterization with *Pseudomonas fluorescens* L 26.1. A: Length averages. B: Length increase % v.s. control

A. Length averages

	Genotype		
Treatment	T5	T6	T10
Control	4.32	4.04	4.22
10^4 c.f.u./g	5.42**	5.24**	5.31**
10^6 c.f.u./g	5.14**	4.79**	5.34**

Notes: Values (cm) with ** are significantly different at P = 0.01 from the non bacterized plants by Duncan's test

B. Length increase % v.s. control



These observations lead to conclude that, in controlled conditions and for a same bacterial root colonization, disparity of susceptibility between certain genotypes is greatly dependent on the plant genome. But for a given genotype it is dependent also on the growth stage. Although the relationship between PGPR such as *Pseudomonas fluorescens* L26.1 and host-plant is commensalistic, the response to the PGPR effect could be controlled by "host susceptibility genes" as for the plant diseases. We suggest these host plant genes play a leading part in the response. A more precise demonstration regarding to the detection and the mode of action of these genes would be of great interest from a scientific and agronomic point of view, because it would allow to analyse and to understand better the fundamental mechanisms of the plant growth promoting effect.

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