PLANT GROWTH-PROMOTING ACTIVITY OF *BACILLUS SUBTILIS* AF1

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ABSTRACT

An antibiotic-producing strain of *Bacillus subtilis* (marked with streptomycin resistance), antagonistic to many plant pathogenic fungi, was used in seed bacterization studies with some economically important crop plants. *B. subtilis* AF1 increased germination, root length, shoot height and dry weight of the test seedlings. The bacterium colonized the root surface when applied with methyl cellosolve at 10^7/CFU/seed. The population of *B. subtilis* AF1 was high on cucumber roots and least on the cotton roots. The bacterium showed a persisting growth-promoting effect on all the test seedlings.

INTRODUCTION

Bacterial strains isolated from the rhizosphere hold great promise as seed innoculants in innovative agricultural systems to promote plant growth and yield. These bacteria are termed as plant growth-promoting rhizobacteria (PGPR), or only 'rhizobacteria', to accentuate their intimate association with roots. Studies with PGPR indicate that the root microflora can profitably be manipulated through use of bacteria which live as epiphytes on roots and beneficially modulate the ecological niche[1,2]. The direct application of micro-organisms to seeds or other plant parts gives them a competitive advantage over pathogens that must compete for nutrients and sites for attachment prior to infection.

Routine use of biological systems in controlling plant diseases has become more attractive due to the added benefit of enhanced plant growth besides disease control. Such enhancement has been achieved with *Bacillus subtilis*[3], fluorescent pseudomonads[2,4,5] and mycoparasites like *Trichoderma harzianum*[6]. Our earlier work[7,8] projected *B. subtilis* AF1 as a potential antagonist of some vascular wilt fungi. In the present study we have examined the use of AF1 as seed treatment for some crop plants viz. cotton, cucumber, pigeon pea, tomato and egg plant, with a view to examine the growth-promoting activity and root-colonizing ability of this antagonist.

MATERIALS AND METHODS

The organism

B. subtilis AF1, isolated from the rhizosphere of pigeon pea plants in wilt non-conducive soils near Bharuch, Gujarat, was found to be a potential antagonist of vascular wilt fungi[7,8] and the streptomycin (100 $\mu g\ ml^{-1}$) resistant mutant of AF1 was used for seed treatment.

Seed bacterization with AF1

Seed bacterization was done by the method of Weller and Cook[9]. Seeds of cotton, cucumber, pigeon pea, tomato and egg plant were surface-sterilized with 2.4% sodium hypochlorite for 3 min, rinsed in distilled water and finally dried overnight under sterile air stream. *B. subtilis* AF1, marked with streptomycin resistance was grown on potato dextrose agar for 48 h at 28 ± 2°C. Bacterial cells from each plate were then scraped from the plates with a sterile glass rod into 5 ml of 1% methyl cellosolve and mixed with 5 g of the test seeds. The treated seeds were placed in sterile petri plates and dried overnight under sterile air stream. Care was taken to avoid clumping of seeds prior to planting.

To estimate the colony-forming units (CFU) of the bacterial suspension used for the seed treatment, coated seeds were macerated in phosphate buffer (pH 7.2) and sampled for CFU on streptomycin-containing nutrient agar. It worked out to be 10^7 CFU/seed. The treated seeds were sown in unsterilized soil-containing earthen pots and kept in green house at 28 ± 2°C. The data on percentage germination, root length, shoot height and dry weight were recorded against controls (treated with sterile methyl cellosolve). Four replicates of 20 each were examined and their mean values were recorded. The root and shoot lengths and dry weight were recorded for 7, 14 and 21 day old seedlings.

Root colonization study

Roots of seedlings were sampled on 7th, 14th and 21st day for the colonizing bacterial population. Plants were dug with a shovel and carried to laboratory in polyethylene bags. The soil adhering to the roots was gently teased and the roots were severed from the seedlings. Rhizosphere soil particles tightly attached to the roots were left behind with the roots. The roots were cut into 1 cm pieces, weighed (100 mg) and dipped in 5 ml of 0.01 M phosphate buffer (pH 7.2). The suspension was shaken thoroughly for 5 min to release the adhering bacteria into the buffer. Appropriate dilutions of the root wash were plated on nutrient agar with streptomycin and the colonies were counted after 48 h of growth.

RESULTS

Effect of seed bacterization on seed germination (figure 1)

A significant increase in the emergence of treated seedlings after 5 days of soaking is evident. Cotton and tomato showed highest inducement (95 and 91% respectively). Cucumber, which showed lowest stimulus in germination, nonetheless, had 28% more germination. *B. subtilis* AF1 thus proved a definite stimulant of seed germination.

Effect of seed bacterization on plant growth

Root length

Seed bacterization augmented the root length as well as shoot height in all the test seedlings (table 1).

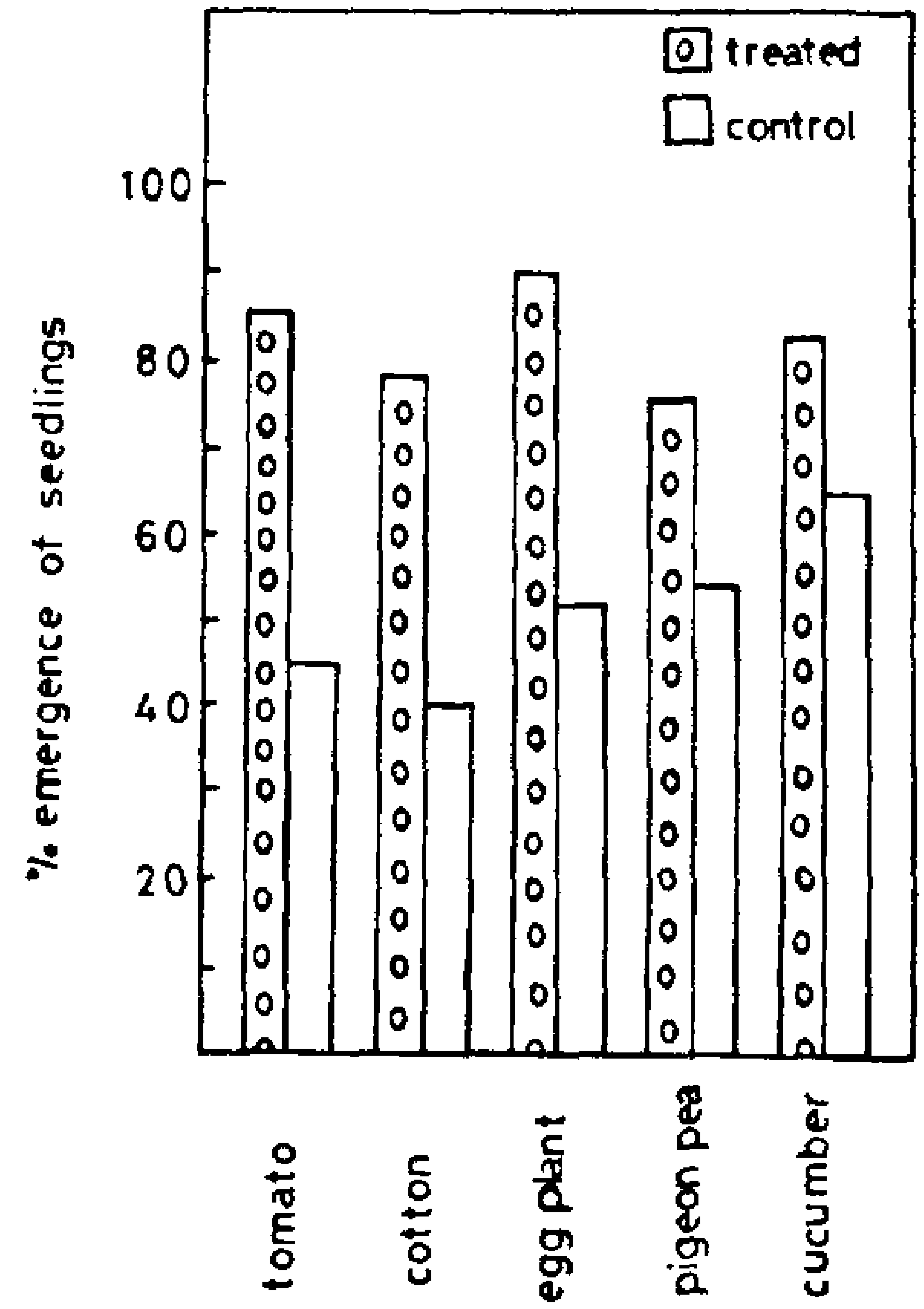

Figure 1. Seed bacterization effect of *Bacillus subtilis* AF1 on the emergence of some economically important crop plants.

Table 1 *Increase in root and shoot length of B. subtilis AF1 treated seedlings over a period of 21 days**

	Cotton	Cucumber	Pigeon pea	Egg plant	Tomato
Root length (in mm) after:					
7 days					
Control	76.5±1.6	36.0±0.9	10.8±0.4	4.6±0.2	1.4±0.1
Treated	81.5±1.4	41.6±1.2	22.4±0.6	9.0±0.4	3.8±0.1
14 days					
Control	84.5±1.4	42.8±0.9	17.6±0.6	8.4±0.3	2.3±0.1
Treated	91.6±1.5	50.6±0.8	31.4±0.6	15.6±0.4	5.2±0.2
21 days					
Control	92.3±1.8	48.0+0.6	26.7±0.5	10.8±0.2	3.6±0.1
Treated	100.4±1.6	58.6±0.4	40.3±0.4	18.4±0.2	7.4±0.1
Shoot length (in cm) after:					
7 days					
Control	8.3±0.2	9.4+0.2	7.6±0.1	4.5±0.1	4.8±0.2
Treated	11.8±0 2	11.3±0.3	10.2±0.2	6.0±0.2	6.9±0.2
14 days					
Control	16.3±0.2	17.5±0.2	10.8±0.3	8.1±0.2	8.3±0.1
Treated	22.4±0.2	24.6±0.3	14.6±0.3	11.8±0.2	11.6±0.2
21 days					
Control	24.5±0.3	28.4±0.3	14.3±0.3	11.8±0.2	12.4±0.2
Treated	31.3±0.3	36.4±0.4	18.6±0.4	14.6±0.3	15.8±0.3

*Mean value of 4 replicates of 20 each.

A steady increase in root length over the control up to 21 days for all the treated seedlings is evident. Also, the differences between control and the treated seedlings increased with the age of the seedlings indicating a persistent effect of bacterial treatment.

Shoot length

The data on shoot height (table 1) attained by the treated seedlings also show an augmented effect as compared to the control. This occurred steadily up to 21 days of seedling growth. The increase in plant height was the highest in cucumber (8 cm) followed by cotton (6.8 cm), pigeon pea (4.3 cm), tomato (3.4 cm) and egg plant (2.8 cm) after 21 days. The seed treatment thus had maximum growth-promoting effect on cucumber and minimum on egg plant, out of the five test seedlings.

Dry weight

Bacterization of seeds with *B. subtilis* AF1 significantly stimulated the weight of the seedlings within 21 days. Compared to the controls a significant increase in the dry weight of all the test seedlings was noticed after 7, 14 and 21 days (table 2). The increase in the dry weight was the highest (37%) in cucumber seedlings followed by tomato (32%), pigeon pea (31%), egg plant (29%) and cotton (26%).

Distribution of bacteria on the root surface

The data presented in table 3 show the distribution of *B. subtilis* AF1 on the roots of the crop plants grown from bacterized seeds (10^7 CFU/seed). The bacteria were detectable on the roots of all the treated seedlings. An increased population of *B. subtilis* AF1 was noted with the age of the seedlings.

Table 3 *Population of B. subtilis AF1 on the roots of some test crop plants after 7, 14 and 21 days of seed bacterization (10^7 CFU/seed)**

Seeds	Population of *B. subtilis* AF1 after		
	7 days	14 days	21 days
Cotton	4.36×10^2	7.58×10^2	1.82×10^3
Cucumber	7.24×10^3	1.14×10^4	1.50×10^4
Pigeon pea	1.20×10^3	1.74×10^3	7.24×10^3
Egg plant	3.47×10^3	5.24×10^3	6.31×10^3
Tomato	1.82×10^3	3.31×10^3	4.37×10^3

*The data represent colony-forming units, mean of three replicates.

The bacterial population was highest on roots of cucumber seedlings (1.50×10^4) and least on cotton (1.82×10^3) roots.

DISCUSSION

The results indicate that *B. subtilis* AF1 colonized the root surfaces and promoted the growth of the test seedlings. The per cent germination of the seedlings was higher in bacterized seedlings as compared to the methyl cellosolve-treated controls. *In vitro* antibiotic-producing property is shared by many rhizosphere bacteria and thus could not be taken as the criterion for selecting PGPR[2]. However, the significant growth increase caused by *B. subtilis* AF1 in seedlings produced from treated seeds definitely qualifies it as a PGPR. Earlier, Broadbent *et al*[10] and Merriman *et al*[3] reported *B. subtilis* A13 (a wide-spectrum antibiotic-producing isolated) as a plant growth-promoting bacterium. Thus, although antagonism *per se* does not qualify an organism as a PGPR, it does not exclude it from the same. The present isolate of *B. subtilis* is antagonistic (fungistatic) to many plant pathogens[11]

Table 2 *Dry weight of the test seedlings inoculated with B. subtilis AF1 over a period of 21 days*

Seeds	Dry weight of the seedlings over a period of								
	7 days			14 days			21 days		
	Control	Treated	% increase*	Control	Treated	% increase	Control	Treated	% increase
Cotton	113	130	15	188	224	19	221	279	26
Cucumber	76	92	21	89	117	31	146	204	37
Pigeon pea	51	64	23	70	88	26	96	126	31
Egg plant	28	35	22	43	54	26	74	98	29
Tomato	28	34	21	38	48	26	66	87	32

*% Increase was calculated as treated − Control/Control × 100.

and at the same time a PGPR. *B. subtilis* AF1 thus holds promise in the newly emerging biotechnology aimed at providing not only biological protection to roots from pathogens but also in augumenting the growth of crop plants.

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ANNOUNCEMENT

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As part of its silver jubilee celebrations the Indian Institute of Tropical Meteorology, Pune is planning to hold the above symposium from 23 to 28 November 1988. This symposium is co-sponsored by a number of national and international scientific organisations including the World Meteorological Organization. About 40 invited papers from leading scientists in India and abroad are expected to be presented and discussed on the following topics: 1. Monsoon modelling and prediction; 2. Summer monsoon systems and dynamics; 3. Winter monsoon systems and dynamics; 4. Monsoon variability; 5. Medium and long-range forecasting; 6. Dynamics of large-scale circulations over the Indian Oceans; 7. Tropical cyclones, and 8. Large-scale air-sea interactions and ocean-atmosphere modelling.

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