THE USE OF PLANT GROWTH PROMOTING BACTERIA FOR IMPROVEMENT THE PLANT GROWTH OF WHEAT, MAIZE AND COTTON IN CALCAREOUS SOIL OF UZBEKISTAN

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Abstract

The objectives of our research were to isolate native plant growth-promoting bacteria from the calcareous soil, to study their effect on plant growth of wheat, maize and cotton under condition of salinated soil of Uzbekistan. The experiments carried out in greenhouse with pot experiments using calcareous soil. The treatments with bacterial strains *Microbacterium M12M, Bacillus sp. B10M, Pseudomonas sp.P29M, Pseudomonas sp. P12M* significantly increased the shoot and root growth of crops. Some of bacterial strains were found to have in vitro antagonistic activity against *Fusarium oxysporum* and *F. solani.* They are salt tolerant and heat resistance. In general our results showed that bacterial inoculation may improve wheat, maize and cotton growth in calcareous soil.

Introduction

Inappropriate application of mineral fertilizers in cotton production in a semi arid region of Uzbekistan has resulted in pollution and salinization of agricultural lands and water resources. The use of a non-hazardous biological method, like bacterial inoculants, in such regions to increase plant production is an important approach to help sustainable development. In particular, plant growth-promoting bacteria (PGPR) have been reported to be key elements for plant establishment under nutrient-imbalance conditions. Their use in agriculture can favour a reduction in agro-chemical use and support ecofriendly crop production (1, 2, 3). PGPR can help the improvement of plant growth, plant nutrition, root growth pattern, plant competitiveness, and responses to external stress factors. They can also inhibit soil borne plant pathogens by producing growth-promoting chemical substances and inducing plant resistance (4, 5, 6, 7). Different plant-growth promoting rhizosphere bacteria, including associative bacteria such as *Azospirillum*, *Bacillus*, *Pseudomonas, Enterobacter* group have been used for their beneficial effects on plant growth (8). Several studies clearly showed the effect of plant growth-promoting bacteria on growth of different crops at different climates, soils and temperatures (9, 10, 11, 12, 13).

However, research to determine the effects of PGPR isolated from the Calcisol soil of Uzbekistan on the growth of cotton, wheat and maize in semi arid environment has not been performed. The major objectives of our research were to isolate the bacterial strains from calcareous Calcisol soil and determine their effect on plant growth of cotton, wheat and maize under conditions of high temperature and nutrient deficient soil.

Materials and methods

The soil for pot experiments was collected from a non-fertilized field site near Tashkent, located in the northeastern part of Uzbekistan. The soil chemical and physical properties are presented in Table 1.

Table 1. Soil chemical properties, and soil particle distribution of the 0-30 cm soil layer

Soil type	C_t	N_t	P	K	Mg	pH	soil particle distribution, %		
	---------mg · 100g $^{-1}$--------						2 – 0.2 mm	0.2 – 0.02 mm	< 0.02mm
Calcisol	200	6	3.0	12.0	6.0	8.5	2.2	54.5	43.3

The total carbon content, C**,** was identified by elemental analysis, while total nitrogen content, N, was determined by the Kjeldahl method. The molybdenum blue method was used to determine the total phosphorus content, P, in soil. Potassium, K, was determined using the Flame Photometric Method (14). The Atomic Absorption Spectrophotometer (AAS) was employed to measure calcium chloride ($CaCl_2$) and extractable magnesium (15). Soil pH-value was measured by means of an electrometer. Soil particle distribution was determined using natrium phosphate**.**

Cotton, wheat and maize were employed in the inoculation experiments. Plant seeds were obtained from the Tashkent University of Agriculture. The bacterial strains *Microbacterium M12M, Bacillus sp. B10M, Pseudomonas sp.P29M, Pseudomonas sp. P12M* were used for this study. The bacterial strains were obtained from the rhizosphere of maize. Glycerin-peptone-agar medium used for isolation of bacterial strains The identification of strains relied on standard biochemical and physiological tests according to the classification of Bergey. Bacterial strains also tested for their antagonistic activity**.** *Fusarium solani and Fusarium oxysporum* were used as indicator strains for antagonistic bacteria. Salt tolerance was determined in Hirte agar medium containing NaCl at 7%.
The study of the effect of isolated strains on plant growth was carried out in pot experiments using a nutrient-poor calcareous Calcisol. The inoculation treatments were set-up in a randomised design with six replicates. The day before sowing, pots were filled with 350 g soil. Three seeds of cotton, maize and four seeds of wheat were sown per pot. After germination, plants were thinned to two per pot. The bacteria were grown in glycerine-peptone-medium. Tubes were secured on a rotary shaker (120 rpm; 23°C) and agitated for three days. Seedlings of these plants were inoculated with 1 ml of the bacterial suspension, which resulted in an inoculum's density of ca. 10^6 cfu/ml. Plants were grown in pots for four weeks under greenhouse conditions with a temperature of 26°C to 30°C during the day and 16°C to 18°C at night. The soil was moistened with water and maintained at 60% of its moisture holding capacity (MHC). Four weeks after germination, shoots and roots were separated and dried at 105°C before determining the root and shoot dry weight. The criteria for growth promotion were studied as root and shoot dry matter and plant length.
The data were analysed with an ANOVA and Student-Newman-Keuls test for testing the significant differences ($p<0.05$) of main effects.

Results

After inoculation of bacterial strains the root and shoot dry weight of maize, wheat and cotton increased compared to the uninoculated plants.
Plant length of maize after inoculation increased up to 85 %, and dry matter increased up to 63%. (Table 2).

Table 2. The effect of Plant Growth Promoting Bacteria on plant length and root, shoot dry matter of maize (Pot experiments, 6 replication, Conbtrol=100%)

Bacterial strains	Plant Length		Dry matter	
	Shoot	Root	Shoot	Root
Control	100 (24,8)[1]	100 (16,3)[1]	100 (124,8)[2]	100 (101,7)[2]
Microbacterium M12M	104	112	139*	132*
Bacillus sp. B10M	140*	179*	163*	127*
Pseudomonas sp.P29M	117	185*	138*	122*
Pseudomonas sp. P12M	135*	185*	143*	115

[1]cm, [2]mg,
*Significantly different from the control for $P < 0.05$

Our experiments with wheat showed that Plant growth promoting bacterial strains effects on plant length positively. They increased shoot and root length from 5 to 43 %.
The bacterial strains increased dry matter of root more that dry matter of shoot of wheat. (Table 3) Root dry matter increased up to 75%. But inoculation did not effect positively to shoot growth.
They had positive effect on shoot and root length. Shoot length increased up to 43 % to compare to the control plants.

Table 3. The effect of Plant Growth promoting bacteria on plant length and dry matter of wheat, (Pot experiments, control =100%, 6 replication).

Bacterial strains	Plant Length		Dry matter	
	Shoot	Root	Shoot	Root

Control	100 (17,9) [1]	100 (12,8) [1]	100 (28,4) [2]	100 (12,8) [2]
Microbacterium M12M	123	105	103	102
Bacillus sp. B10M	129*	125*	108	124*
Pseudomonas sp.P29M	127*	109	109	141*
Pseudomonas sp. P12M	143*	115*	107	113

[1]cm, [2]mg
*Significantly different from the control for P < 0.05

Our results with cotton showed that bacterial strains have effect on plant length and dry matter positively. (Table 4).

Table 4. The effect of Plant Growth promoting bacteria on plant length and dry matter of cotton, (Pot experiments, control =100%, 6 replication).

Bacterial strains	Plant Length		Dry matter	
	Shoot	Root	Shoot	Root
Control	100 (5,7) [1]	100 (5,2) [1]	100 (28,4) [2]	100 (12,8) [2]
Microbacterium M12M	105	105	106	180*
Bacillus sp. B10M	122*	134*	145*	167*
Pseudomonas sp.P29M	121*	167*	119*	195*
Pseudomonas sp. P12M	129*	125*	138*	198*

[1]cm, [2]mg
*Significantly different from the control for P < 0.05

Root dry matter of cotton increased up to 98 %. The bacterial inoculation effect positively also to the plant high. *Pseudomonas* sp. P29M were a best isolates among plant growth promoting bacteria.
Also we have tested bacterial strains for their antagonistic activity against plant pathogenic Fungi. As plant pathogenic fungi we have used *Fusarium oxisporum, Fusarium solani*. Our results showed that most bacterial strains were antagonist against *Fusarium oxisporum*.

Discussion

This work demonstrated that independent of the origin, selected growth-stimulating bacteria isolates are able to increase the growth cotton, wheat and maize in nutrient poor Calcisol soil. Our bacterial isolates *Microbacterium M12M, Bacillus sp. B10M, Pseudomonas sp.P29M, Pseudomonas sp. P12M* had a significant effect on cotton, wheat and maize in nutrient-poor Calcisol soil, while non-treated plants by comparison performed poorly under such conditions. The bacterisation only marginally increased yields when tested under ideal climatic situations (16). The greatest benefits occurred when crops encountered stressful conditions for prolonged periods. Our bacteria isolates are temperature resistance and salt tolerant and therefore able to survive in dry hot summer conditions. They are physiologically distinct, suggesting adaptation to their respective environmental conditions. In another works (12, 13) also were found he bacteria, which stimulated the plant growth of wheat and rice in warm climates.
In summary, the final results of the bacterial plant growth-promotion in our experiment show that plant growth-promoting bacteria can play an essential role in helping plants establish and grow in nutrient deficient conditions. From these results we conclude that inoculation of cotton, wheat and maize with effective strains may significantly enhance plant length and growth in pot experiments. The demonstrated results reflect pot experiments with relatively constant soil humidity. Bacterial strains *Rhizobium trifolii* R39 and *Pantoea agglomerans* PF76/4 isolated from soil in Germany increased plant growth in pot experiments and were also effective in field experiments (17). However, the extent of stimulation of plants by bacteria and their persistence in plant growth-promotion activity under actual field conditions in Uzbekistan remains unclear. The experiments concerning stimulation of cotton, wheat and maize by effective bacterial strains must be followed by investigations under field conditions.

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