IMPROVEMENT OF WHEAT AND COTTON GROWTH AND NUTRIENT UPTAKE BY PHOSPHATE SOLUBILIZING BACTERIA

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ABSTRACT

Pot and field experiments were carried out on calcareous calcisol soil for evaluating the effects of phosphate solubilising bacterial inoculants combined with phosphorit on wheat, maize and cotton growth and yield. Stimulatory effects of bacterial species such *Pseudomonas, Bacillus, Arthrobacter* and *Rhizobium* on growth of wheat, maize and cotton growth, yield, N, P –uptake, soil P content were recorded. The results revealed that plant growth promoting bacteria combined with phosphorit significantly increased shoot, root length of wheat and maize. The phosphorus content was significantly increased in cotton plants inoculated with *Rhizobium meliloti* combined with phosphorit with respect to the uninoculated plants growing in the control soil. Standard treatment without bacterial inoculation has resulted very low P uptake in plants. This result suggests that phosphate solubilising bacteria are able to mobilise more P to the plants and improve plant growth.

INTRODUCTION

Phosphorus is a important element for growth development and yield of many crops. However many soils throughout the world are P-deficient because the free phosphorus concentration even in fertile soils is generally not higher than 10 μM even at 6.5 where it is most soluble (Arnou, 1953). Phosphorus deficiencies are common nutritional problems in crop production also in Uzbekistan.

Soil microorganisms have enormous potential in providing soil phosphates for plant growth. Phosphorus biofertilizers in the form of microorganisms can help in increasing the availability of accumulated phosphates for plant growth by solubilisation (Goldstein, 1986; Gyaneshwar et al., 2002). In addition, the microorganisms involved in P solubilisation as well as better scavenging of soluble P can enhance plant growth by increasing the efficiency of biological nitrogen fixation, enhancing the availability of other trace elements and by production of plant growth promoting substances (Gyaneshwar et al., 2002). Application of phosphorites along with phosphate solubilising bacteria (PSB) improved P uptake by plants and yields indicating that the PSB are able to solubilise phosphates and to mobilise phosphorus in crop plants (Rogers, 1993). In this respect, biofertilisation technology has taken a part to minimise production costs and at the same time, avoid the environmental hazards (Galal et al., 2001). Phosphorus application and bacterial inoculation affect yield of soybean through their effects on phosphorus use efficiency (Shah, 2001). Also they are successful applied in the cultivation of barley and chick pea plants (Rodriguez-Barraeco, 2002). A P-solubilizing *Rhizobium leguminosarum* has been shown to increase the growth of maize and lettuce (Chabot et al., 1996). The PSB- plant inoculations resulted in 10-15% increases in crop yields in 10 out of 37 experiments (Tandon, 1987). These studies also demonstrated an increase in P

uptake by plants. There are only a few reports of P solubilization by Rhizobium (Chabot et al., 1993). In this study, the effect of new biopreparation based on PSB bacteria, *Rhizobium meliloti* on plant growth of wheat, maize and nutrient uptake and yield of cotton grown in P deficient soil were investigated.

MATERIAL AND METHODS

Soil and Plants

The soil for pot experiments was collected from a non-fertilized field site near Tashkent, located in the northeastern part of Uzbekistan. Soil is calcareous serozem soil (1 % organic matter, 0.6 mg N 100 g-1 soil; 3.0 mg P 100 g $^{-1}$; 12 mg K 100 g^{-1}; 6 mg Mg 100 g^{-1} soil; pH 7.4) having a calcic horizon within 50 cm of the surface. The orchic horizon is low in organic matter. The climate is continental with mean annual rainfall of 200 mm. For pot experiments the soil sampled from the surface orchic horison (0-30 cm). 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 (Riehm 1985). The Atomic Absorption Spectrophotometer (AAS) was employed to measure calcium chloride ($CaCl_2$) and extractable magnesium (Schachtschnabel and Heinemann. 1974). Soil pH-value was measured by means of an electrometer. Soil particle distribution was determined using natrium phosphate**.** Wheat, maize, **c**otton were employed in the inoculation experiments. Plant seeds were obtained from the Tashkent University of Agriculture.

Microorganisms

Bacterial strains *Pseudomonas* sp. RM3M, *P. denitrificans* PsD6, *P. rathonis* PsR47, *Bacillus laevolacticus* BcL28, *B. amyloliquefaciens* BcA27, *Arthrobacter simplex* ArS43, and *Rhizobium meliloti* were used for the experiments. Glycerin-peptone-agar medium used for isolation of bacterial strains (Hirte, 1961). For isolation of rhizosphere bacteria 1 g washed roots of wheat, and maize was macerated and shacked with 10 ml sterile water. The resulting suspensions were evaluated for colony forming units (cfu) according to the dilution-plate method in glycerine-peptone-agar. With the addition of TMTD, the native fungal and bacterial flora was largely excluded from the plates. After an incubation time of 7 days at 28° C the reisolated, strains were identified. The identification of strains relied on standard biochemical and physiological tests according to the classification of Bergey (Holt et al., 1994). Gram stain, morphology, spore formation, motility, nitrate reduction, and gas production from glucose were determined according to methods for LAB described by Gerhardt (1981). Salt tolerance was determined in Hirte agar medium containing NaCl at 7%.

Plant Growth and Inoculation in Pots

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 wheat, and maize 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. Additionally bacterial strains applied to the plants with combination phosphorit. Plants were grown in pots for four weeks under greenhouse conditions with a temperature of 26°C to 28°C during the day and 17°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 determined the root and shoot length.

Field Experiments

The field trials were conducted at the experimental farm of Institute of organic and inorganic chemistry, Uzbekistan. Recommended rates of phosphorus (140kg P h^{-1}, as phosphorit and superphosphate), nitrogen (200 kg N $ha^{-1,}$ as ammonium sulphate) and potassium (60 kg K $h^{-1,}$ as potassium sulphate) were applied. Treatments were: plants without treatments 1. (NoPoKo), 2. ($NP_{superphosphate}K$), ($NP_{phosphorit}K$), ($NP_{phosphorit+PSB}K$). These treatments were distributed in a randomised complete block design with four replications. The plot size was 5 m by 3 m. Cottonseeds were obtained from the University of Agriculture, Tashkent. *Rhizobium meliloti* URM1 used as phosphate soulubilisng bacterial inoculant, which combined with phosphorits (inoculum density 10^9 cells g^{-1}). Plants were harvested at tillering, flowering and maturity stages. Dry matter accumulation, N, P uptake efficiency in plants have been determined.

Statistical Analysis

The data were analysed with an ANOVA and Student-Newman-Keuls test for testing the significant differences ($p<0.05$) of main effects.

RESULTS AND DISCUSSION

Bacterial inoculation affected the early plant growth of wheat and maize. Many of our bacterial strains *Bacillus*, *Pseudomonas* and *Arthrobacter* had a significant effect on growth of wheat, maize in nutrient-poor Calcisol soil, while non-treated plants by comparison performed poorly under such conditions. Defreitas (1992) also demonstrated that in low fertility Asquith soil, *Pseudomonas* bacterial strains significantly enhanced early plant growth. According to Lazarovitz and Nowak (1997), the bacterisation only marginally increased yields when tested under ideal climatic situations. The greatest benefits occurred when crops encountered stressful conditions for prolonged periods.

After inoculation of bacterial strains combined with phosphorit the root and shoot length of maize, and wheat increased compared to the uninoculated plants. (Fig. 1, 2). Plant length of wheat after inoculation increased up to 22 %. The most effective shoot and root length promoting isolate was *Arthrobacter simplex* ArS43, which generated 22% increase in shoot length of plant and 17% root growth. over the control (Fig.1). Chaykovskaya 2001) reported, that PSB increased Phosphorus accumulation in plants, yield of pea and barley. The bacterial strains were able dissolve hard soluble organophosphates. Inoculation also lead to the increase of N content in the biomass of both plants. Jumaniyazova et al., (2002) reported that PSB *Bacillus* sp. mobilize phosphate from organic hard soluble phosphoric compounds and increased growth and yield of cotton in Calcisol soil.

Our experiments with maize showed that plant growth promoting bacterial strains effects on plant length positively. They increased shoot length up to 53%. (Fig.2). Most effective bacterial strains was *Ps. rathonis* PsR47 and *B. amyloliquefaciens* BcA27, which increased root growth up to 20% compare control plants. The combination of bacterial strains with phosphorit has lower effect on plant growth to compare single bacterial inoculation.(Fig. 2). According Asea et al., (1988) Bacillus megatherium is considered the most effective PSMs according to field experiments.

Field Experiments

The inoculation with phosphate solubilising bacteria also positively effected on shoot root growth of cotton in field experiments. The results of our experiments showed, that PSB combined with phosphorit have a significant effect on dry matter accumulation in leaves, shoot and root (Table 2). Compared to the control and fertiliser used along, the PSB combined with phosphorit was superior over the other treatments. Higher effect was found in maturity stage. In tillering stage of cotton, bacterial inoculation did not effect significantly. Co-inoculation of *Azospirillum, Rhizobium* and *Azotobacter* with PSMs showed synergistic effect on plant growth and crop yields (Barea et al., 1975).

In field experiments all treatments increased yield of cotton in comparison to control plants (Fig 1). Higher yield obtained after treatment with PSB *Rhizobium meliloti* URM1. The yield of cotton increased up to 77% (285.7 g^{-1} plant). PSMs can also increase the growth of plants by mechanisms other than P solubilisation, e.g. production of phytohormones such as Indole acetic acid (Arshad and Frankenberger, 1998). According to the results obtained, PSB was able to mobilise phosphorus efficiency in cotton. The phosphorus content was significantly increased in cotton plants with treatment PSB combined phosphorit (Table 3). The standard treatment with fertiliser along did not effect P uptake in plants. Shah et al., (2001) also reported phosphorus uptake efficiency and yield increased with phosphorus application and with inoculation.

A positive influence of treatments on soil P content is marked (Table 3). Soil P content in the variant with PSB reaches 6.0 mg $P_2O_5.100^{-1}$ soil. It has been found that application of phosphorit combined with PSB leads to the increase of P content in soil (tillering, flowering and maturity stages of Plants).

In summary, the final results of the bacterial plant growth-promotion in our experiments show that plant growth-promoting and phosphate solubilising bacteria can play an essential role in helping plants establish and grow in nutrient deficient conditions. PSB are able to mobilise more P into plants, where hard soluble phosphates are presented in soil and increased yield and growth.

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Table 1. The effect of Posphate solubilizing bacteria (PSB) *Rizobium meliloti* URM1 combined with phosphorite on dry matter of cotton (field experiments, g.plant $^{-1}$)

Treatments	Tillering		Flowering			Maturity		
	leaves	stem	leaves	stem	bud	leaves	Stem	bud case
NoPoKo	8.1	7.0	46.5	10.5	15.5	54.6	39.5	35.3
$NP_{superp}K$	9.5	6.3	47.0	18.7	17.1	53.0	42.1	36.0
$NP_{phosphorite}K$	8.9	6.0	46.9	18.0	18.4	57.4	51.0	38.3
$NP_{PSB}K$	14.6	8.7	47.0	18.9	23.4	89.1	64.8	49.5

Table 2. The effect PSB combined with phosphorite on N and P uptake of cotton (field experiments, N and P content in %)

Treatments	Leaves		Stem		Bud case		Cotton fibers	
	N	P	N	P	N	P	N	P
NoPoKo	1.45	0.51	0.68	0.21	0.78	0.19	1.78	0.81
$NP_{superp}K$	1.55	0.75	0.75	0.24	0.83	0.22	1.87	0.84
$NP_{phosphorite}K$	1.2	0.2	0.3	0.1	0.5	0.1	1.6	0.4
$NP_{PSB}K$	1.62	0.8	0.75	0.24	0.83	0.25	1.9	0.89

Table 3. Phosphorus content in soil as affected by PSB combined with phosphorite (before sowing 1.8 mg P_2O_5 . 100^{-1} soil)

Treatments	Tillering	Flowering	Maturity
NoPoKo	2.4	1.5	2.2
$NP_{superp}K$	2.8	6.0	4.7
$NP_{phosphorite}K$	2.0	1.8	1.9
$NP_{PSB}K$	5.4	6.0	4.0

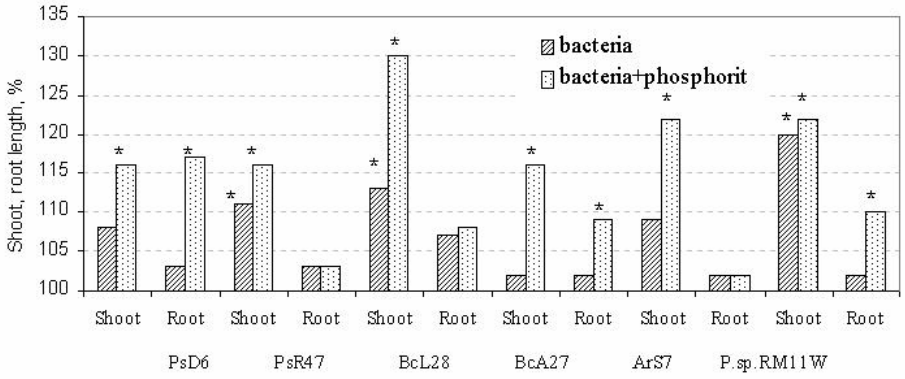

Fig. 1. The effect of plant growth promoting bacteria combined with phosphorit on shoot and root length of wheat in pot experiments (control=100%).

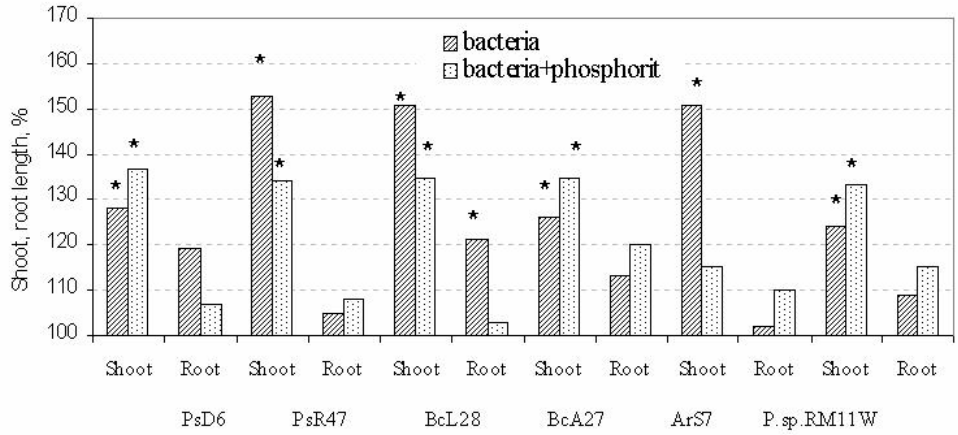

Fig. 2 The effect of plant growth promoting bacteria combined with phosphorit on shoot and root length of maize in pot experiments (control=100%).

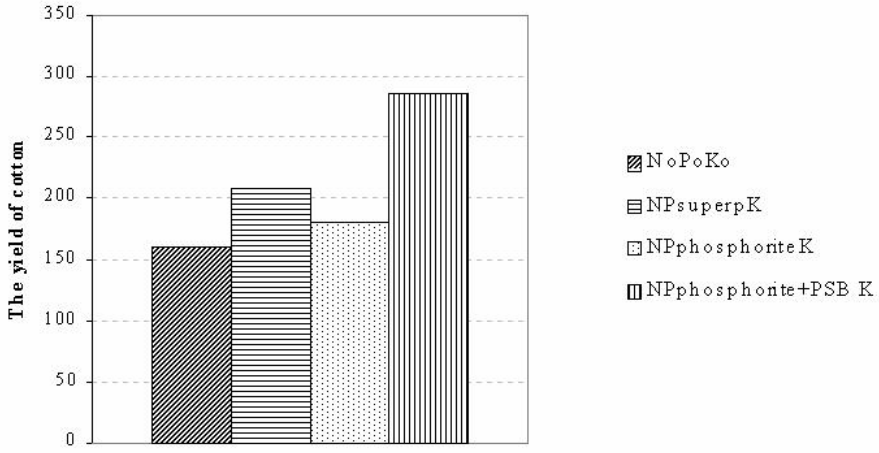

Fig.3 The effect of PSB combined with phosphorite on cotton yield in field experiments, g^{-1} plant, (Control plants, 160 g^{-1} plant =100%)