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Performance of MPS Bacterial Inoculation in Two Consecutive Growth of Maize Plants

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Abstract : Two successive *in vitro* experiments were carried out to examine the effect of MPS bacterial inoculation on growth, and nitrogen and phosphorus accumulation of maize plants under greenhouse condition in the same soil. There were four treatments, uninoculated control and three phosphate solubilizing bacterial inoculations, *viz., Pseudomonas striata, Burkholderia cepacia* and *Serratia marcescens*. The inoculated plants showed the higher plant height, total dry mass, nitrogen and phosphorus accumulation when compared to uninoculated control plants in both experiments. In the combined data analysis from two experiments, the plants inoculated with *P. striata* and *B. cepacia* showed significantly higher plant height, total dry mass and P accumulation when compared to *S. marcescens* inoculated plant and uninoculated control plants. The *P. striata* and *B. cepacia* inoculation enhanced total dry matter accumulation by 14% and phosphorus accumulation by 25% over the uninoculated control plants. The nitrogen and phosphorus concentration of maize plants were also increased due to MPS bacterial inoculation, however, the effect was not significant.

Key words : maize, phosphobacteria, phosphate, *Burkholderia cepacia, Pseudomonas striata, Serratia marcescens.*

INTRODUCTION

Phosphorus is one of the essential element in crop production and is major plant nutrient required in sufficient amounts for higher crop yield as well for proper functioning of soil biota. The P content in average soils is about 0.05% (w/w) and only 0.1% of the total P is available to plants[1)]. Its cycle in the biosphere described as open or sedimentary, because there is no interchange with the atmosphere[2)]. Microoanisms play a central role in the natural phosphorus cycle. A number of soil microorganisms is capable of dissolving insoluble inorganic and organic phosphate to orthophosphate ions (H_2PO_4 and HPO_4)[1)]. These free inorganic P ions in soil solution can be assimilated in appreciable amounts by plants[3,4)]. The major microbiological processes of P solubilization were due to acidification, chelation and exchange reaction in growth environments[5)]. Several reports have examined the ability of different bacterial species to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium pre-phosphate, hydorxyapatite, rock phosphate, iron and aluminum phosphates[6,7)]. As phosphate compounds in Korean soils are predominantly inorganic, chiefly locked as $AlPO_4$ and $FePO_4$[5)], the group of phosphate solubilizing microorganisms dissolving insoluble phosphate seem to have implication in Korean agriculture. During last decade researchers have tried to increase plant available P by means of phosphate solubilizing microorganisms[8-10)].

The bacterial genera such as *Pseudomonas, Bacillus, Burkholderia, Aachromobacter, Agrobacter, Flavobacter* and *Erwinia* has been reported to have P-solubilizing activity[11)]. Inoculation with P-solubilizing microorganisms have shown to increase the P uptake and yield of several crop plants[12-14)]. There have been a number of reports on growth promotion by bacteria that have the ability to solubilize inorganic and/or organic P from soil after their inoculation in soil or plant seeds[4,15,16)]. Simultaneous increases in P uptake and crop yield have also been observed after inoculation with *Bacillus* spp.[17,18,)]. *The Pseudomonas putida* also stimulated the growth of shoots and increased ^{32}P-labled phosphate uptake in canola[19)]. The *Pseudomonas striata* strain showing phosphate solubilizing ability significantly improved grain and dry matter yields with concomitant increase in

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P uptake[20].

Phosphate solubilizing bacteria play an important role in plant nutrition through the increase in P uptake by the plant, and their use as PGPR is an important contribution to agricultural crops. Further investigations are needed to improve the performance phosphate solubilizing bacteria as bacterial inoculants. In this study two consecutive experiments were conducted to examine the effect of MPS bacterial strain inoculations on plant growth, and N and P accumulation in the maize plant.

MATERIALS AND METHODS

Mineral phosphate solubilizing (MPS) bacterial strains *viz.*, *Pseudomonas striata, Burkholderia cepacia* and *Serratia marcescens,* were obtained from the Department of Agricultural Microbiology, UAS, Dharwad, India. All the strains maintained in nutrient agar slants.

The inoculum of each bacterium was prepared separately by transferring a loopful of 48 h old culture to 50 mL nutrient broth. After two days of incubation at 28℃, the entire broth culture was transferred to a 1-L Erlenmeyer flask containing 500 mL nutrient broth. The flasks were incubated at 28°C for 5 days. The standard population of each MPS bacterial was 10^8 CFU/mL, which was made after serial dilution plate methods and inoculated to soil.

Soil was collected from the agricultural farm of Chungbuk National University. The initial chemical characteristics of the soil were determined in accordance with the standard methods[21] (Table 1). The soil was autoclaved twice with 7 days interval, and the pots (10x10 cm) were filled with 500 g of autoclaved soil. After completion of the first experiment, the same soil of each treatment and replication retained and it was used for the second experiment.

Seeds of maize (*Zea Mays*) were purchased from a market. The seeds were presoaked in water for 1 day before sowing. Three seeds per pot were dibbled into the soil. Thinning was done to maintain 1 seedling per pot 15 days after sowing.

The first experiment was conducted without any fertilizer application. However, in the second experiment the recommended dose of N and K were applied to each pot, but P was not given to any treatments. The pots were watered periodically to maintain the moisture to field capacity in each pot. Plant height, dry matter, N and P uptake were measured at 45 days after sowing by following standard methods. The plants

Table 1. P status, organic matter content and pH in soil used in this study

pH	Organic matter	Ava.-P	Al-P	Fe-P	Ca-P	Total-P
	g/kg	········	········	mg P_2O_5/kg	········	········
5.51	8.79	433.96	377.44	239.89	102.93	1688.90
(±0.01)	(±0.69)	(±4.64)	(±12.32)	(±13.11)	(±5.78)	(±7.22)

sample were dried and ground to powder using Wiley mill in order to estimate the N and P concentrations.

Nitrogen and P concentration were measured using the standard Kjeldahl method[22] and ammonium molybdate-ascorbic acid method,[23] respectively. Nitrogen and phosphorus accumulation was calculated by multiplying the dry mass and concentrations of N and P, respectively.

Results of the present experiment were statistically analyzed as completely randomized design using software package DRYSOFT DESIGN.

RESULTS AND DISCUSSION

Although several phosphate-solubilizing bacteria occur in soils, usually their population is not high enough to compete with other bacteria commonly established in the rhizosphere. Therefore inoculation of plants by a target P solubilizing bacteria at a higher rate is necessary to explore its beneficial trait for sustainable crop production.

The plant heights were not significantly different among the MPS bacterial inoculations in the first experiment. However, all the inoculated plants showed higher plant height compared to the uninoculated control plants (Table 2). In the second experiments, the plant height was significantly increased due to the three MPS bacterial inoculations. The *P. striata* inoculated plants showed the highest plant height, which was significantly superior over the all other inoculated plants. However, *B. cepacia* inoculated plants also showed the higher plant height than the *S. marcescens* inoculated plants. Combined analysis of the both consecutive growth experiments also indicated that the significant increase in plant height is due to MPS bacterial inoculation. The highest plant height was recorded in the plants inoculated with *P. striata,* which was significantly higher than

Table 2. Effect of MPS bacterial inoculation on plant height and total dry mass of maize plants in two consecutive growth

Inoculation	Plant height			Total dry mass		
	1st Expt	2nd Expt	Mean	1st Expt	2nd Expt	Mean
	cm			g/plant		
Serratia marcescens	91.0	80.3	85.7	3.21	2.33	2.77
Burkholderia cepacia	91.3	87.2	89.3	3.46	2.84	3.15
Pseudomonas striata	97.25	95.8	96.5	3.37	3.07	3.22
Uninoculated Control	76.2	73.3	74.8	2.71	2.09	2.40
$LSD_{0.05}$	NS	5.97	6.07	0.25	0.22	0.20

Table 3. Effect of MPS bacterial inoculation on nitrogen and phosphorus accumulation of maize plants in two consecutive growth

Inoculation	N - Accumulation			P - Accumulation		
	1st Expt	2nd Expt	Mean	1st Expt	2nd Expt	Mean
	mg/plant			mg/plant		
Serratia marcescens	24.0	26.2	25.1	1.2	0.5	0.8
Burkholderia cepacia	26.2	33.5	29.9	1.4	0.8	1.1
Pseudomonas striata	24.4	32.0	28.2	1.2	0.7	1.0
Uninoculated Control	23.0	24.6	23.8	1.0	0.4	0.7
$LSD_{0.05}$	NS	2.13	1.90	NS	0.17	0.06

other MPS bacteria inoculated plants. The least plant height resulted in the plants without inoculation in all the cases.

The plants inoculated with MPS bacterial strains, showed significantly higher shoot dry mass when compared to the uninoculated control plants (Table 2). The dry masses of *B. cepacia* and *P. striata* inoculated plants were not significantly different, however, both of which were found to be significantly superior over *S. marcescens* inoculated and uninoculated control plants in the first experiment. In the second experiment, *P. striata* inoculated plants showed higher dry mass when compared to all other inoculated plants. The least dry mass was recorded in the uninoculated control plants in both consecutive growth experiments. In the pooled analysis of two consecutive growth experiments, *P. striata* and *B. cepacia* inoculated plants showed significantly higher dry mass than that of plants inoculated with *S. marcescens*. The uninoculated plants showed the lowest dry mass. However, dry masses of *P. striata* and *B. cepacia* inoculated plants were not significantly different. Increase in plant height and dry mass due to MPS bacterial inoculation was also reported in other field crops[24-26].

The increased plant height and dry mass in inoculated plants attributed to the increased phosphate accumulation in the maize plants of MPS bacterial inoculation (Table 3).

The plants inoculated with *B. cepacia* and *P. striata* accumulated significantly higher amount of P than *S. marcescens*. The least P accumulation was recorded in the plants devoid of inoculation. Phosphorus accumulation in *P. striata* inoculated plant was lower compared to *B. cepacia* inoculated plants, however, plant height and dry mass were higher in *P striata* inoculated plants. This might be due to the plant growth substance production by *P. striata*. Similarly, increased growth in *P. striata* inoculated sunflower plants was attributed to its PGPR activity[15]. The *Burkholderia cepacia* inoculated plants also displayed significantly higher mineral phosphate solubilization and moderate phosphatase activity[12].

Maximum N accumulation was found in the plants inoculated with MPS bacteria when compared to plants without inoculation (Table 3). The plants inoculated with *P. striata* and *B. cepacia* were not significantly different with respect to N accumulation. However, both of these strains were significantly superior over *S. marcescens* inoculated plants and uninoculated plants. The least N accumulation was found in the uninoculated plants. Similarly, higher N accumulations in MPS bacterial inoculated plants were reported in other crop plants[15,26]. The increase in N accumulation due to inoculation can be ascribed to increased P availability to the crop. Phosphorus is known to increase the root growth and proliferation by creating more absorptive area for uptake of other nutrient especially N.

The shoot P concentration of maize plant was increased due to the MPS bacterial inoculation, however the increase was not significant in the both experiments (Table 4). The mean shoot P concentration of the second experiment was reduced and this is mainly due to the experiments conducted without application of P fertilizer.

In the two separate consecutive experiments of MPS bacterial inoculation to maize plants, the first experiments resulted higher plant height, dry mass, and N and P accumulation when compared to the second experiment. These results are mainly due to the experiments conducted without application

Table 4. Effect of MPS bacterial inoculation on nitrogen and phosphorus concentration of maize plants in two consecutive growth

Inoculation	N - Concentration			P - Concentration		
	1st Expt	2nd Expt	Mean	1st Expt	2nd Expt	Mean
	---- mg N/GDW ----			---- mg P/GDW ----		
Serratia marcescens	6.11	11.02	8.57	0.33	0.18	0.26
Burkholderia cepacia	6.39	11.79	9.09	0.35	0.20	0.28
Pseudomonas striata	6.94	10.63	8.79	0.34	0.22	0.28
Uninoculated Control	6.04	10.44	8.24	0.32	0.16	0.24
$LSD_{0.05}$	NS	0.2	NS	NS	NS	NS

of P fertilizer to know the P solubilizing efficiency of the bacterial strains. The reduction of these parameters might indicate that the proper application of P is necessary, which reveals about integrated nutrient managements. Hence, the combined use of chemical fertilizer and biofertilizer has been considered as a workable technology. The MPS bacterial inoculation in combination with 75% recommended dose of single super phosphorus or 100% recommended dose of rock phosphorus plus MPS bacterial inoculation gave the same yield as that of 100% recommended dose of single super phosphate without inoculation[15].

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