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SHORT COMMUNICATION

ESTABLISHMENT AND INFLUENCE OF PHOSPHATE SOLUBILIZING BACTERIA ON PEARL MILLET

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Two Phosphate solubilizing bacterial isolates and their *lac*Z marked transconjugants were checked for their establishment in the rhizosphere and response on pearlmillet under pot house condition. Seed bacterization showed establishment of PSB in the rhizosphere upto 60 days after sowing. The counts were slightly higher in inoculated treatments compared to uninoculated control. Application of rock phosphate and phosphatic fertilizers increased the biomass by 35-50 per cent over control. The P-uptake also improved with soil amendments and seed inoculation at both stages of sampling.

***Key words: Lac*Z strains, pearl millet, phosphate solubilizing bacteria (PSB), plant biomass.**

Phosphorous is the major nutrient of all living systems due to its essential physiological roles in cellular metabolism. Plants absorb inorganic phosphorous which exists at a very low concentration in the soil due to its fixation with other minerals. Microbes are known to solubilize insoluble phosphates by the production of organic acids, siderophores, chelating compounds. Seed bacterization with phosphate solubilizing bacteria (PSB) improved crop yield (Venkateshwarlu *et al*. 1984, Yadav and Dadarwal 1997, Kannaiyan *et al.* 2000, Sunita 2002). The possible reasons for increase in yield may be due to release of P and other nutrients, phytohormone production, antagonism to plant pathogens and promotion of plant growth promoting rhizosphere microorganisms. However, the establishment of such microorganisms in the rhizosphere is not clearly known so far. The major problem for such a study has been lack of suitable and simple methodology for detection of introduced bacteria. The use of antibiotic markers and techniques like ELISA are very laborious, costly, insensitive with a lower detection limit and incapability of distinguishing between viable and non viable cells (Mc Cormick 1986, Ford and Olson 1988, Hurse and Date 1992 and Garg *et al*. 1995). On the other hand, introduction of foreign genes such as *lac*Z, *gus*A or *gfp* which can be easily detected on a chromogenic substrate is a quick and reliable method for identification of introduced bacteria (Kamboj *et al.* 1996, Sharma *et al*. 1997, O' Kane *et al.* 1998 and Pal *et al*. 2000). This technology can help in monitoring the response of bioinoculants by facilitating the identification of introduced microorganisms. The work was therefore planned to understand the establishment of PSB (marked with *lacZ* gene) in pearl millet (*Pennisetum americanum*) rhizosphere and to study their response on plant biomass under pothouse conditions.

Two PSB strains (44R and A6) and their transconjugants (44RM and A6M) derived by conjugation of PSB with *E.coli* S17-1 (pTn-B20) were obtained from the culture collection of the Department of Microbiology, CCSHAU, Hisar, Haryana. All the strains were maintained in the refrigerator on Pikovskaya's medium (Pikovskaya 1948) by transfers at regular intervals.

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Sandy loam soil of the following composition (pH, 7.8, organic carbon, 0.12%, total nitrogen, 0.04%, available P, 2.5 ppm) was used @ 5 kg/pot for pot house experiment with pearl millet. Different doses of single super phosphate (SSP) and Mussoorie rock phosphate (MRP) @ 20 kg and 40 kg P_2O_5 ha^{-1}, respectively were mixed in the upper 5 cm layer of the soil. The soil also received urea @ 20 kg N ha^{-1} as basal dose. Seeds of pearl millet bio-var. HHB-44 were sterilized and treated with PSB cultures containing approx. 10^8 cells ml^{-1}. Initially ten seeds were sown per pot. Ten days after sowing (DAS), 3 plants per pot were retained. There were six pots for each treatment. The following sixteen treatments were kept for the study of inoculation response on pearl millet: Control, 44R, 44RM, A6, A6M, MRP-40kgP_2O_5, 44R+MRP, 44RM+MRP, A6+MRP, A6M+MRP, SSP-20kg P_2O_5, 44R+ SSP, 44RM+SSP, A6+SSP, A6M+SSP and SSP-40 kgP_2O_5 ha^{-1}. Sampling was done at 30 and 60 DAS for PSB count and plant biomass. Total number of bacteria, PSB and marked strains were enumerated, on nutrient agar, Pikovskaya's medium and Pikovskaya's medium containing kanamycin (50 µg ml^{-1}), nalidixix acid (25 µg ml^{-1}) and X-gal. The plant biomass was determined after drying the samples overnight 80°C, respectively to a constant weight.

The total phosphorous content of pearl millet shoots was estimated by a modified colorimetric method using ascorbic acid as described by John (1970). The concentration was calculated using standard curve prepared with P (0.1 to 1 µg ml^{-1}).

The inoculation studies on use of phosphate solubilizing microorganisms have been encouraging but their establishment in the rhizosphere was not clearly known. The population of introduced strains needs monitoring for correlating the response of crops to the introduced bacteria and to reveal the reasons for such an effect,. Earlier, it was difficult due to lack of suitable methodology as the techniques available were very complicated and costly. For the present investigation, studies were conducted with strains marked with *lac*Z to monitor their survival in the rhizosphere and to check their response under pot house conditions. Inoculation response of PSB isolates 44R, A6 and their transconjugants 44R M and A6M in combination with varying amounts of SSP and MRP was checked on pearl millet crop. Total bacterial and PSB count was determined by dilution plating on Pikovskaya's medium with and without antibiotics.

The results showed that the total bacterial and PSB counts were higher in the inoculated treatments compared to the uninoculated control at both the stages (30 and 60 DAS) of plant growth, however, it was more at later stage (Table 1). This shows the survival of introduced bacteria in the rhizosphere even upto 60 DAS. The counts were also higher in the treatments supplemented with MRP and SSP over the control showing that soil amendments also helped in the improvement of bacterial number in the rhizosphere due to availability of phosphorous. Similar results were also reported by Venkateshwarlu *et al*. (1984). The PSB count was approx. 100 times less than the total bacterial counts indicating that about 1% of total bacteria have the ability to solubilize P. The establishment of transconjugants in the soil was confirmed by growing them on plates containing X-gal and antibiotics (Kan 50 & Nx 25). It was found that blue coloured colonies appeared only on plates receiving soil samples from treatment with the transconjugants. The presence of transconjugants was also shown by Pal *et al*. (2000). The number of transconjugants was 10% of total PSB count suggesting that apart from the introduced transconjugants, certain indigenous bacteria were already present in the soil. The introduction of PSB increased the number of P solubilizers which in return helped the plant growth. Similar results have also been reported by Sunita (2002) on mungbean.

The plant biomass was also recorded at the two stages (30 and 60 DAS) of plant growth to examine the effect of PSB and inorganic P. Increase in plant biomass was observed at both stages with seed bacterization. However, there was no significant difference among cultural treatments. The treatments with transconjugants (either alone or in combination with MRP or SSP) resulted in slightly higher plant biomass as compared to respective controls. The application of SSP showed a significant increase in plant biomass over control which was further improved with seed bacterization. It was interesting to note that MRP together with PSB gave plant response close to 20 kg P_2O_5 ha^{-1} as SSP which

Table 1. Total bacterial and PSB count in pearl millet rhizosphere at different stages of plant growth.

Treatments	Total bacteria ($x10^6$ cfu/g)		PSB ($x10^4$ cfu/g)		Transconjugants ($x10^2$ cfu/g)	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
Control	17	25	18	20	ND	ND
44R	19	28	20	23	ND	ND
44RM	31	36	30	28	9	10
A6	20	22	32	32	ND	ND
A6M	27	25	30	27	5	8
20kg MRP	17	17	19	25	ND	ND
20kg MRP+44R	20	25	17	32	ND	ND
20kgMRP+44RM	15	20	20	40	7	14
20kg MRP+A6	18	24	60	57	ND	ND
20kg MRP+A6M	22	32	50	54	5	7
20kg SSP	28	34	21	30	ND	ND
20kg SSP+44R	40	42	23	28	ND	ND
20kg SSP+44RM	36	38	30	36	17	14
20kg SSP+A6	27	57	30	36	ND	ND
20kg SSP+A6M	22	48	54	58	17	15
40kg SSP	25	32	15	27	ND	ND

ND: Not detected

suggests use of these organism with rock phosphate. Similar results were also reported by Yadav and Dadarwal (1997).

The results indicate that the application of PSB increased plant biomass but these strains can be used as potential bioinoculants only if they facilitate the uptake of phosphorous by plants. Quantitative estimation of total phosphorous uptake in pearlmillet shoots was therefore carried out. The results showed a slight increase in phosphorous in all the treatments compared to control (Table 2). Seed inoculation with A6 in combination with MRP or SSP at 30 DAS showed higher uptake of phosphorous indicating its role in solubilization of phosphorous and making it available to the plants. P-uptake further increased at 60 DAS as compared to 30 DAS due to increase in plant biomass. The PSB are potential agents for crop production as also reported by Kannaiyan *et al*. (2000).

The results show survival of PSB in the soil upto 60 DAS which help in plant growth and P-uptake in pearlmillet. MRP with PSB gave a response comparable to half the recommended dose of phosphorous as SSP. The data suggests the use of *lac*Z marker as a potential tool in understanding the establishment of introduced bacteria and for explaining the contributions of such organism in plant growth.

Table 2. Effect of PSB inoculation on plant biomass and P-uptake by pearl millet under pot house conditions

Treatments	Root weight (g/plant)		Shoot weight (g/plant)		P-uptake (mg/plant)	
	30 DAS	60 DAS	30 DAS	60 DAS	30 DAS	60 DAS
Control	0.41	1.26	0.83	1.94	1.83	5.63
PSB-44R	0.50	1.49	0.88	2.30	2.11	6.90
PSB-44RM	0.49	1.89	0.75	2.13	2.10	6.80
PSB-A6	0.45	1.80	0.99	2.02	2.48	6.26
PSB-A6M	0.47	1.47	1.02	2.64	2.96	8.98
40kg P_2O_5 ha^{-1} MRP	0.40	1.27	0.82	2.29	2.05	7.09
40kgMRP+44R	0.46	1.64	0.87	2.39	2.44	8.13
40kgMRP+44RM	0.54	1.51	1.05	2.68	3.15	9.38
40kgMRP+A6	0.46	1.58	1.16	2.45	3.13	8.58
40kgMRP+A6M	0.47	1.59	1.06	2.33	3.07	8.62
20kg P_2O_5 ha^{-1} SSP	0.56	1.78	1.11	2.31	3.11	7.85
20kgSSP+44R	0.58	1.82	1.16	2.59	3.36	9.07
20kgSSP+44RM	0.65	2.00	1.39	2.71	4.45	10.03
20kgSSP+A6	0.66	2.15	1.30	2.50	3.90	9.50
20kgSSP+A6M	0.68	2.31	1.58	2.29	5.37	8.93
40kg P_2O_5 ha^{-1} SSP	0.63	1.89	1.69	2.86	5.58	10.87
CD at 5%	0.21	0.68	0.49	0.71	0.39	0.92

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