J. Mycopathol. Res. **40**(2) : 201–203 (2002)

Influence of combined inoculation with *Rhizobium* and phosphobacteria on mungbean in field

A. CHATTERJEE AND P. BHATTACHARJEE
Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur 741252, West Bengal

Effects of inoculation with *Rhizobium* sp. and phosphate solubilizing bacteria of mungbean [*Vigna radiata* (L) Wilczch] variety B-1, on nodulation and grain yield were studied. Field trials were conducted taking eight treatments which were replicated four times in a randomised block design with 6 sq. m. net plot size. Results from the field trial revealed that the plant inoculated with *Rhizobium* strains and phosphate solubilizing bacteria of seed showed increased rate of nodulation, N-content and grain yield. Per cent increase of grain yield over control was observed to be higher significantly in the plants when *Rhizobium* strains JCa-1 and M-10 were treated at seed along with phosphobacterial culture containing *Bacillus polymyxa* and *Pseudomonas striata.*

Key Words : *Rhizobium* sp., *Bacillus polymyxa, Pseudomonas striata,* combined inoculation, mungbean.

INTRODUCTION

Research in the last few decades has established that phosphobacteria can improve plant growth through increased phosphate solubilization and uptake of phosphorus. Moreover on the basis of available literature, recently reviewed by Subba Rao (1984), on the existence of associative effects betweent differen P-solubilizers and N_2 fixers under natural condition, it was rightly suggested that dual effect of P-solubilizing microorganisms and specific N_2 fixing rhizobia can be exploited for better nitrogen and phosphorus nutrition of the legumes. In this context, the present investigation has been undertaken in order to study the response of mungbean inoculated with strains of *Rhizobium* (JCa–1 & M–10) and phosphate solubilizing microbes containing *Bacillus polymyxa* and *Pseudomonas striata,* in different combinations with reference to nitrogen and phosphorus nutrition of the plants.

MATERIALS AND METHODS

Field trials were conducted for mungbean (*Vigna radiata*) variety B-1, with eight different treatments in a randomised block design with 6 sq.m. net plot size in the University Farm in two consecutive *kharif* seasons. The soil of the emperimental plot was sandy loam. Seeds of the host plant were inoculated with charcoal based inoculum of the respective symbiont cultures, namely JCa–1 & M–10 strains of cowpea group *Rnizobium* at a strength of 28.80×10^6 cells/ml and 32.66×10^6 cells/ml. Phosphate solubilizers containing *B. polymyxa* and *Ps. striata* at a strength of 7×10^8 cells/ml at the time of sowing. *Rhizobium* cells were grown in Yeast Mannital Agar (YMA) medium (Vincent, 1970) which was prepared without agar agar. Phosphate solubilizing bacteria were grown on Pikovskaya broth (Pikovskaya, 1948). Total N-content of the dried plant sample was estimated following the methods as described by Jackson (1962).

Observations were made on 45 days after sowing in terms of nodulation (number and weight) and N-fixation on sample plants @ 5% of the plants population for estimation of nodulation. Yield of mungbean were estimated by harvesting and weighing of grains obtained per plot and represented as q/ha.

RESULTS AND DISCUSSION

(i) Effect on nodulation

Results presented in Table 1 revealed that the plants inoculated with the combined strains of *Rhizobium* namely JCa–1 and M –10 along with phosphobacteria had increased rate of nodulation. The number of nodules were estimated about 37.50 and 38.26 in the 1st and 2nd year respectively as compared to control. The rate of increase was 117.39% and 87.73% in the 1st and 2nd year respectively over control. In case of combined inoculation of *Rhizobium* strains, nodules to the tune of about 35.75 and 35.80 were obtanined in two consecutive years i.e. increase @ 101.40% and 75.66% in the 1st and 2nd year respectively over control.

As regards nodule fresh weight, the significant increases were recorded where the plants were inoculated with the combined strains of *Rhizobium* along with phosphae solubilizing bacteria at seed. In this case the nodule fresh weights were recorded to the tune of 0.16 g and 0.23 g in two consecutive years.

(ii) Effects on total nitrogen content of plants

Results presented in the Table 2 revealed that nitrogen content of the mungbean plants increased due to inoculation with any of the test micro-organism over the uninoculated control. As expected *Rhizobium* had the best effect in this regard. However, nitrogen content of the plant increased considerably when they were subjected to combined inoculation with strains of *Rhizobium* and phosphate solubilizing bacterium at seed. The increase wes 0.59% in the 1st and 0.45% in the 2nd year respectively.

(iii) Effect on grain yield

Yield content in mungbean was found to be augmented in the presence of phosphobacterium (Table 2). However, response to combined inoculation with the strains of *Rhizobium* and phosphobacterium at seed was not only the maximum among different used but the same was found to be significantly of higher order than that of *Rhizobium* inoculation.

The rate of increase over control were recorded to be significantly higher in the plants when they were subjected to combined inoculation with strains of *Rhizobium* and phosphobacterium at seed (R_1+R_2+ PSB+seed), which was 122.95% in the 1st year and 141.76% in the 2nd year respectively followed by the plants treated with strains of *Rhizobium* (JCa–1+M–10) at seed, where the rate of increase over control were 78.92% in the 1st year and 64.95% in the 2nd year respectively.

It was interesting to note that the *Rhizobium* application was best in combination with seed rather than with seedlings after 15 DAS and/or 30 DAS (Subba Rao and Tilak 1977 ; Patra and Bhattacharyya, 1998). Bhargava *et al.* (1974) reported that there was better nodulation and higher leghemoglobin content in the nodule in case of plants with *Rhizobium* inoculated at seed.

It is well known that a leguminous rhizosphere encourages proliferation of the growth of its own rhizobia. But in the present investigation the mungbean rhizosphere appeared to be congenial for the growth of PSB as well.

Table 1 : Effect of inoculation of mungbean with *Rhizobium* sp. and PSB on nodulation in field at 45 DAS.

Treatments	Nodule number/plant		Per cent increase or decrease over control		Fresh weight of nodules (g)//pant	
	Mean				Mean	
	X	Y	X	Y	X	Y
Uninoculation control	17.75	20.38			0.06	0.04
R_1+Seed	30.75	35.20	73.23	72.71	0.07	0.06
R_2+Seed	30.75	33.50	73.23	64.37	0.11	0.13
PSB+Seed	22.25	28.75	25.35	41.06	0.06	0.04
R_1+PSB+Seed	32.75	29.00	84.50	42.29	0.13	0.14
R_2+PSB+Seed	36.25	37.81	110.14	85.52	0.15	0.19
R_1+R_2+Seed	35.75	35.80	101.40	75.66	0.15	0.17
R_1+R_2+PSB+Seed	37.50	38.26	117.39	87.73	0.16	0.23
CD at 5%	9.66	13.55			0.029	0.049
CD at 1%	12.70	17.81			0.038	0.065
S EM±	13.95	19.56			0.042	0.071

R_1– JCa–1 strain of cowpea *Rhizobium*, R_2–M–10 strain of cowpea *Rhizobium*

PSB– Phosphate solubilizing bacteria (combination of *B. polymyxa* and *Ps. striata*)

X = First year; Y = Second year, DAS = Days after sowing.

It is quite likely that the presence of rhizobia in the

legume rhizosphere influence the legume roots to secrete growth promoting substances which in turn might have enhanced the growth of PSB in field. The most interesting feature, which has emerged out of the present investigation is that a synergistic effect may be achieved in case of double inoculation.

Table 2 : Effect of inoculation of mungbean with *Rhizobium* sp. and PSB on N-content and yield in field condition.

Treatments	Mean yield q/ha		Per cent increase or decrease over control		N content (%) of the Plant	
	X	Y	X	Y	X	Y
Control	8.54	9.10	–	–	0.012	0.016
R_1+Seed	13.76	11.82	61.12	29.89	0.21	0.19
R_2+Seed	13.08	14.33	53.16	57.47	0.26	0.28
PSB+Seed	13.01	11.01	52.34	20.99	0.14	0.12
R_1+PSB+Seed	10.71	14.87	25.41	63.41	0.26	0.27
R_2+PSB+Seed	17.71	17.92	107.38	96.92	0.33	0.34
R_1+R_2+Seed	15.28	15.01	78.92	64.95	0.26	0.28
R_1+R_2+PSB+Seed	19.04	22.00	122.95	141.76	0.59	0.45
CD at 5%	2.39	2.83			0.115	0.998
CD at 1%	3.14	3.72			0.151	0.131
S EM±	3.45	4.09			0.166	0.144

R_1– JCa–1 strain of cowpea *Rhizobium*
R_2–M–10 strain of cowpea *Rhizobium*
PSB– Phosphate solubilizing bacteria (combination of *B. polymyxa* and *Ps. striata*)
X = First year; Y = Second year, DAS = Days after sowing.

Effectiveness of legume *Rhizobium* symbiosis are reported to be improved in presence of a free living nitrogen fixing or a P–solubilizing micro-organism (Shende *et al.*, 1973). The PSB used in this investigation, was not only capable of solubilizing a good amount of insoluble phosphate but can also fix nitrogen in free living state. Hence it is reasonable to believe that its dual property might have been well exploited in improving the symbiotic efficiency of mungbean. Moreover it is well know that in P–depleted soil legume fails to establish a good symbiotic relationship with the rhizobia (Demeterio *et al.*, 1972) and that phosphate solubilizing bacteria and mycorrhizal infection leads to better nodulation and nitrogen fixation by the legumes (Poi *et al.*, 1989).

In this sub continent, the average yield of the pulses are far below the world average. It is, in this context, quite appropriate to think of sooting up of the pulse yields by effective use of the lwo cost biofertilizers, in combined forms, at the same time utilizing available large deposits of rock phosphates. This would not only help to improve soil fertility in a better way but would also help to fill up the protein gap, now being seriouslly experienced by the third world.

REFERENCES

Bhargava, J. K., Saxena, M. C. and Tilak, KVBR (1974). Response of soybean to inoculation with various cultures of *Rhizobium japonicum. Proc. India. Nat. Sci. Acad.*, **40**(5) : 471-475.

Demeterio, J. L., Ellis. R. Jr., and Paulsen, G. M. (1972). Nodulation and Nitrogen fixation by two soybean varieties as affected by phosphorus and zinc nutrition. *Agron. J.* **64** : 566.

Jackson, M. L. (1962). *Soil chemical analysis*. Prentice Hall. Inc. Eaglewood; Cliffs; N. J.

Patra, D.K. and Bhattacharyya, P. (1998). Response of cowpea rhizobia on nodulation and yield of mungbean (*Vigna radiata* L. Willczek). *J. Mycopathol. Res.* **36** (1) : 17–23.

Pikovskaya, R. L. (1948). *Microbiologiya*. **17.** 362.

Poi, S. C., Ghosh, G. and Kabi, M. C. (1989). Response of chickpea (*Cicer arietinum* L.) to combined inoculation with *Rhizobium,* Phosphobacteria and Mycorrhizal organisms. *Zentralbl Mikrobiol.* **144** : 249–253.

Shende, S. T., Arora, C. K., and Sen, A. (1973). Interaction between *Azotobacter chroococcum, Bacillus megatherium* ver. *phosphaticum* and *Rhizobium* sp *Zpl. Bakt. 11.* **128** : 668.

Subbs Rao, N. S. (1984). Interaction of nitrogen fixing micro-organism with other soil microorganisms in *Current development in Biological Nitrogen Fixation* (Ed. N. S. Subba Rao). Oxford and IBH Pub. New Delhi 37–64.

Subba Rao, N. S. and Tilak, K. V. B. R. (1977). Rhizobial Culture, their rate in pulse production. Souvenir Bulletin. Directorate of Pulse development, Govt. of India, Lucknow, P. 31–34.

Vincent, J. M. (1970). *A mannual for the practical study of the root nodule bacteria*. IBP Hand Book No. 15. Blackwell and Scientific Pub. Oxford.

(Accepted for publication August 8 2002)