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Original Article

Effect of Rhizobzoteria on *Rhizobium* sp. Strain Competition for Nodulation Sites in Urdbean

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Abstract

Co-inoculation effect of rhizospheric microorganisms viz. N_2 fixer (*Azotobacter chroococcum*), PSB (*Bacillus megaterium* and *B. polymyxa*) and PGPR (*Pseudomonas fluorescens* and *Bacillus sp.*) on occupancy of inoculated *Rhizobium* sp. in root nodules of urdbean were examined in two separate studies under field conditions at Pantnagar. Inoculated *Rhizobium* sp. alone formed 23 to 58 % nodules in competition with native *Rhizobium* at different crop growth stages. Inoculation of *A. chroococcum, B. megaterium and P. fluorescens* with *Rhizobium* sp. significantly increased the percent nodule occupancy of inoculated *Rhizobium* sp., by 13.0 to 38.3, 6.2 to 52.9 and 6.2 19.6 % over *Rhizobium* sp. alone at different intervals, respectively. *Bacillus* sp. and *B. polymyxa* indicated adverse effect on competitive ability of inoculum *Rhizobium* sp.. Combined inoculation of *Rhizobium* sp. + *A. chroococcum* + *Bacillus* sp. and *Rhizobium* sp. + *B. megaterium* + *P. fluorescensb* gave significantly more nodule occupancy, by 14.6 to 54.3 and 11.8 to 66.6 %, and recorded better nodule number and nodule dry weight than *Rhizobium* sp. alone at different intervals.

Keywords

Urdbean; *Vigna Mungo. Rhozobium*; PSB; Rhizobacteria; Strain Competition.

Introduction

Seed inoculation with effective *Rhizobium* at sowing is a recommended agronomic practice for pulse production technology. Albeit, poor to moderate status of root nodulation in urdbean (*Vigna mungo* (L.) Hepper) at farmers field, this crop gives poor and variable response to *Rhizobium* inoculation under field conditions (Khurana *et al*., 1997). It could be attributed to the strong competition between inoculated and native rhizobia for nodulation sites on host. Besides, genetics of the symbionts and environmental factors, rhizospheric microorganisms also influence the competitive ability of inoculant strain(s) Many workers have reported the positive effect of phosphate solublising bacteria (PSB) and plant growth promoting rhizobacteria (PGPR) on legume-rhizobia symbiosis particularly in the early events (Dashti *et al,* 1998; Dube, 1997) and synergism between these organisms might increase competitiveness and efficiency of *Rhizobium* inoculation in pulse crops. The present paper communicates the co-inoculation effects of rhizospheric microorganisms viz. N_2 fixer (*Azotobacter chroococcum*), PSB (*Bacillus megaterium* and *B. polymyxa*) and PGPR (*Pseudomonas fluorescens* and *Bacillus sp.*) on strain competition between inoculated and native *Rhizobium* sp.and nodulation in urdbean under field conditions.

Materials and Methods

Source of Inoculants

An effective strain of urdbean *Rhizobium* (UP-1) having intrinsic resistance of 250 mg / ml streptomycin + 150 mg / ml ampicillin and *Azotobacter chroococcum* (R2) were obtained from Department of Soil Science and PGPR (*Pseudomonas fluorescens*, GRP-3) from Department of Microbiology in the university and their charcoal based inoculants were prepared. PSB inoculants of *Bacillus megaterium* and *Bacillus polymyxa* were obtained from TNAU, Coimbatore and CCSHAU, Hissar, respectively.

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Field Studies

Two field experiments were conducted during *kharif* 1997 and 1999 in sandy loam soil of pH 7.3 and low in organic C, and available N and medium in available N and P. Treatments consisted of inoculation with *Rhizobium* sp., *A. chroococcum* and *Bacillus* sp., either alone or in combinations, and uninoculated control in kharif 1997 and inoculation with *Rhizobium, Bacillus megaterium*, *Bacillus polymyxa* and *Pseudomonas fluorescens*, either alone or in combinations, 40 Kg P_2O_5 / ha as basal through SSP and uninoculated control during kharif 1999 were tested following Randomized Block Design in 4 replications in plot of 2.4 m × 4.0 m size. Urdbean seeds (cv. PU 35) were treated with the required inoculants (20 g / kg seed) at the time of sowing and crop was raised as per the recommended agronomic practices.

Strain Competition

Five plants from the each plot were randomly uprooted along with a soil core at 30, 45 and 60 days after sowing (DAS) during 1997 and at 30, 45 and 60 DAS during 1999. Soil cores with plant roots were placed in sieve and washed off with water jet. Forty randomly selected nodules from each replicate plot were removed and surface sterilized in separate test tubes with 95 per cent ethyl alcohol for 2 to 3 minutes. The nodules were crushed in the same test tubes having 4-ml sterile distilled water with a flame sterilized glass rod. The nodule suspensions were streaked on YEMA plates containing 250 mg / ml streptomycin + 150 mg / ml ampicillin. Each plate containing eight streaks of eight nodules was duplicated and incubated at 28 ± 2°C for 4 days. Per cent nodule occupancy of inoculum *Rhizobium* strain was calculated as described by Chandra and Pareek (1985).

Nodulation

Similarly, another 5 plants from each plot were uprooted at above intervals, roots were washed, nodules were removed, counted and their dry weights were determined after drying to constant weight.

Results and Discussion

Strain Competition

Inoculated *Rhizobium* sp. (UP-1) alone formed 23 to 28 % nodules in competition with native *Rhizobium* at different crop growth stages (Table 1) indicating the presence of highly competitive strain of native rhizobia in the soil. Inoculation of *A. chroococcum* with *Rhizobium* sp. significantly increased the percent nodule occupancy of inoculated *Rhizobium* sp., by 38.3, 20.6 and 13.0 % over *Rhizobium* sp. alone at 25, 40 and 60 DAS, respectively. *Bacillus* sp. in conjunction with *Rhizobium* sp., barring at 40 DAS, did not favour the competitiveness of inoculum *Rhizobium* sp. However, combined inoculation of *A. chroococcum* + *Bacillus* sp. + *Rhizobium* sp. formed significantly more nodules than *Rhizobium* sp. + *A. chrococcum* at 40 and 60 days after sowing. The favourable influence of *A. chroocoocum* on nodule occupancy could be attributed to synthesis of plant growth promoting substances as reported by Pandey and Kumar (1989). In another study, *Rhizobium* sp. alone formed 24.0 to 57.7 % nodules at different growth stages (Table 2). *B. megaterium* and *P. fluorescens* when inoculated with *Rhizobium* sp. increased the percent nodule occupancy significantly, by 6.2 to 52.9 and 6.2 to 14.7 %, respectively, in comparison to *Rhizobium* sp. alone at different crop age suggesting that *B. megaterium* and *P. fluorescens* either favoured the survival of inoculum in rhizosphere or by synthesised plant growth promotory substances leading to more root hair development and rhizobial infection (Yahlom *et al*, 1988). *B. polymyxa* along with *Rhizobium* sp. drastically reduced the occupancy of inoculum *Rhizobium* sp. in nodules due to its harmful effect on *Rhizobium* sp. growth as observed by us under cultural conditions. These results corroborate with the findings of Palzinski and Rolfe (1985). Combined inoculation of *Rhizobium* sp. + *B. megaterium* + *P. fluorescens* recorded maximum occupancy of inoculum in nodules, however, it was significantly better than *Rhizobium* sp. + *B. megaterium* only at 45 days and at par with *Rhizobium* sp. + *P. fluorescens* at different intervals. Inoculation of *P. fluorescens* also alleviated the harmful effects of *B. polymyxa,* when applied with *Rhizobium* sp.

Nodulation

Inoculated *Rhizobium* sp. alone significantly increased the number and dry weight of root nodules over the uninoculated control in both the experiments (Table 3 and 4) which could be attributed to presence of large number of ineffective native *Rhizobium* population in the field. Nodulation response to *Rhizobium* sp. inoculation by urdbean has also been reported by Tripathi *et al*. (1994). *A. chroococcum* alone, compared to the control, produced significantly more nodule dry weight at all the growth stages. However, in conjunction with *Rhizobium* sp. it was at par with *Rhizobium* sp. alone treatment. These results corroborate with the findings of Yadav *et al.* (1994)

who also reported increased nodulation following *Azotobacter* sp. inoculation in chickpea due to secretion of plant growth harmones. *Bacillus* sp. alone, compared to the uninoculated control, did not significantly affect nodulation. Moreover, when co-inoculated either with *Rhizobium* sp. or *A. chroococcum*, it reduced the nodulation significantly compared to their individual performance. The results appear to indicate that probably *Bacillus* sp. is antagonistic to urdbean *Rhizobium* sp. as well as *A. chroococcum* and hence when inoculated alone or with either of the above bacteria it restricted infection by reducing native as well as inoculated *Rhizobium* sp. population. Parmar and Dadarwal (1997) also reported such antagonistic interaction among rhizobia and *Bacillus* sp. Inoculation of all the three inoculants together in the present study, though registered highest number and dry weight of nodules, it was not significantly better than that of *Rhizobium* sp. + *A. chroococcum or* even *Rhizobium* sp. alone treatments, barring one observation at 40 DAS.

In second experiment, both the PSB inoculants were comparable and recorded significantly more number and dry weight of nodules over the control at 30 and 45 days. Favourable effect of both PSB on nodulation appears due to increased P supply as reported also by Singh (1994). Dual inoculation with *B. megaterium* + *Rhizobium* sp. was statistically similar to *Rhizobium* sp. alone in nodulation while *B. polymyxa* + *Rhizobium* sp. recorded significantly less nodule number than *Rhizobium* sp. alone at 30 DAS and nodule dry weight at 45 DAS. Adverse effects of *B. polymyxa* on *Rhizobium* sp. performance were also indicated on number and dry weight of nodules at other growth stages possibly due to its antagonistic interaction with inoculated *Rhizobium* sp. as observed also in strain competition. These results are in conformity of Khurana and Sharma (2000) who also found similar effect of PSB + *Rhizobium* sp. in chickpea. *P. fluorescens* alone favoured the nodulation significantly in comparison to the control at 30 and 45 DAS. *P. fluorescens* + *B. megaterium* also produced significantly more nodules than *P. fluorescens* or *B. megaterium* alone at 30 and 45 DAS. *B. polymyxa* adversely affected the performance of *P. fluorescens* by recording numerically less number and dry weight of nodules than *P. fluorescens* alone. This could be attributed to its harmful effect on *P. fluorescens* like *Rhizoboum*. Combined inoculation of *Rhizobium* sp. + *B. megaterium* + *P. fluorescens* gave the highest number and dry weight of nodules registering significant increase in nodule number over *Rhizobium* sp. + *B. megaterium* or *Rhizobium* sp. + *P. fluorescens* at 30 DAS.

Table 1: Nodule occupancy (%) of *Rhizobium* sp. due to *A. chroococcum* and *Bacillus* sp. inoculation at different intervals

Treatment	Days after sowing		
	25	40	60
Rhizobium sp.	25.3	28.2	23.0
Rhizobium sp. + A. chroococcum	35.0	34.0	26.8
Rhizobium sp. + Bacillus sp.	26.8	33.3	24.3
Rhizobium sp. + A. chroococcum + Bacillus sp.	29.0	40.0	35.5
C.D. at 5%	4.0	4.9	2.8

Table 2: Nodule occupancy (%) of *Rhizobium* sp. due to *B. megaterium, B. polymyxa* and *P. fluorescens* inoculation at different intervals

Treatment	Days after sowing		
	30	45	45
Rhizobium sp.	24.0	57.7	53.0
Rhizobium sp. + B. megaterium	36.7	64.2	56.3
Rhizobium sp. + B. polymyxa	19.3	25.	31.0
Rhizobium sp. + P. ?uorescens	27.5	69.0	56.3
Rhizobium sp. + B. megaterium+P. ?uorescens	40.0	75.3	59.3
Rhizobium sp. + B. polymyxa+P. ?uorescens	23.3	63.3	49.3
C.D. at 5%	7.2	9.3	10.5

Table 3: Effect of *Rhizobium* sp., *A. Chroococcum* and *Bacillus* sp. inoculation on urdbean nodulation at different plant growth stages

Treatment	Nodule number/ Plant			Nodule dry weight (mg/ plant)		
	25 DAS	40 DAS	60 DAS	25 DAS	40 DAS	60 DAS
Uninoculated	13.0	25.0	12.3	10.7	19.6	9.9
Rhizobium sp.	18.0	29.8	15.3	16.4	25.3	14.3
A.chroococcum	13.3	26.8	13.0	15.2	23.5	11.7
Bacillus sp.	12.0	23.3	11.8	11.1	21.2	10.7
Rhizobium sp. + *A. chroococcum*	18.8	31.0	15.8	18.1	26.6	14.9
Rhizobium sp.+ *Bacillus* sp.	16.5	26.0	13.8	12.4	20.4	12.2
A. chroococcum + *Bacillus sp.*	13.3	25.0	12.3	12.0	20.4	11.2
Rhizobium sp.+*A. chroococcum* + *Bacillus* sp.	20.3	31.8	16.0	19.7	28.0	15.2
CD at 5%	2.5	2.8	2.7	2.4	2.5	1.5

DAS = Days after sowing

Table 4. Effect of *Rhizobium*, PSB and PGPR on urdbean nodulation at different growth stages

Treatment	Nodule Number/Plant			Nodule dry weight mg/plant)		
	30 DAS	45 DAS	60 DAS	30 DAS	45 DAS	60 DAS
Uninoculated	16.0	14.4	2.6	6.4	15.7	5.3
Rhizobium sp.	25.1	19.5	3.5	10.4	19.4	6.1
B. megaterium	22.4	21.1	3.4	9.4	19.2	5.9
B. polymyxa	20.4	19.3	2.4	9.3	17.5	6.0
P. ?uorescens	20.6	24.3	2.6	9.8	18.0	6.1
Rhizobium sp.+ *B. megaterium*	26.8	22.2	3.5	10.5	20.4	6.4
Rhizobium sp.+ *B. polymyxa*	20.2	23.3	3.2	9.6	17.6	5.6
Rhizobium sp.+ *P. ?uorescens*	22.4	22.2	3.3	9.4	22.1	5.8
B. megaterium P. ?uorescens	27.4	32.6	4.1	9.4	19.6	6.1
B. polymyxa + *P. ?uorescens*	17.8	21.2	3.0	8.4	17.5	5.7
Rhizobium + *B. megaterium* + *P. ?uorescens*	35.7	33.7	5.3	12.2	21.0	7.3
Rhizobium + *B. polymyxa* + *P. ?uorescens*	22.6	23.2	3.6	9.8	18.2	6.5
40 Kg P_2O_5/ ha	21.2	22.4	3.5	11.3	19.0	6.2
CD at 5%	3.0	5.1	NS	2.5	1.3	NS

References

1. Chandra, R. and Pareek, R.P. Role of host genotype in effectiveness and competitiveness of chickpea (*Cicer arietinum* L.) *Rhizobium*. Tropical Agriculture (Trinidad). 1985; 62: 90-94.
2. Dashti, N., Zhang, F., Hynes, R. and Smith, D.L. Plant growth promoting rhizobacteria accelerate nodulation and increase nitrogen fixation activity by field grown soybean (*Glycine max* (L.) Merr.) under short season conditions. Plant and Soil. 1998; 200: 205-213.
3. Dube, S. K. Co-inoculation of phosphate solubilizing bacteria with *Bradyrhizobium japonocum* to increase phosphate availability of rained soybean in Vertisol. Journal of the Indian Society of Soil Science. 1997; 45: 506-509.
4. Khurana, A.S. and Sharma, Poonam Effect of dual inoculation of phosphate solubilizing bacteria, *Bradyrhizobium* sp. (*Cicer*) and phosphorus on nitrogen fixation and yield of chickpea. Indian Journal of Pulses Research. 2000; 13: 66-67.
5. Khurana, A.L., Namdeo, S. L., Patil, B.J., and Dudeja, S.S. On farm experiments on rhizobial inoculants: Problems and possible solutions. In: Extending Nitrogen Fixation Research to Farmers field. (Rupela, O.P., Johansen.C. and Herridge, D.F. Eds). ICRISAT, Patancheru, India. 1997; pp 217-266.
6. Pandey, A. and Kumar, S. Potential of *Azotobacter*s and Azospirilla as biofertilizers for upland agriculture. A review. Journal of Scientific and Industrial Research. 1989; 48: 134-144.
7. Parmar, N. and Dadarwal, K.R. Rhizobacteria from Rhizosphere and Rhizoplane of chickpea (*Cicer arietinum* L.). Indian Journal of Microbiology. 1997; 37: 205-210.
8. Plazinski, J. and Rolfe, B.G. Interaction of *Azospirillum* and *Rhizobium* strains leading to inhibition of nodulation. Applied and Environmental Microbiology. 1985; 35: 199-204.
9. Singh, H.P. Response to inoculation with *Bradyrhizobium, Vesicular arbuscular mycorrhiza* and phosphate solublising microbes on soybean in Mollisol. Indian Journal of Microbiology. 1994; 34: 27-31.
10. Tripathi, M. L., Namdeo, K.N., Tiwari, K.P. and Kurmvansi S.M. Relative efficiency of nitrogen and *Rhizobium* inoculation on growth and yield of *kharif* blackgram. Pulses and Oilseeds. 1994; 7: 328-333.
11. Yadav, K.S., Suneja, S. and Sharma, H.R. Effect of dual inoculation of *Rhizobium* and *Azotobacter* in chickpea (*Cicer arietinum*). Environment and Ecology. 1994; 12: 865-868.
12. Yahlom, R., Okon. Y. and Dovrat, A. Early nodulation in legumes inoculated with *Azospirillum* and *Rhizobium.* Symbiosis. 1988; 6: 69-79.