*Journal of Crop and Weed 4(2) : 21-23(2008)*

# Symbiotic effectiveness of urdbean [*Vigna mungo* (L.) Hepper] through inoculation with *Rhizobium* alongwith co-inoculants

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## ABSTRACT

A three-year field study was conducted during *kharif* season of 2004, 2005 and 2006 at the Pulses and Oilseeds Research Sub-station, Beldanga, Murshidabad, West Bengal,India to study the effect of seed inoculation either singly with *Rhizobium* (CRU 7) or in combination with plant growth promoting rhizobacteria (CRB 1, CRB 2, CRB 3, PUK 46B6, PUK 171 and KB 133) on growth, nodulation and seed yield of urdbean. The results revealed that significantly highest mean seed yield was obtained under co-inoculation with *Rhizobium* + PGPR strain (CRB 2) due to significant increment in most of the parameters studied, as compared to uninoculated control. Yield advantages due to inoculation with *Rhizobium* + PGPR strain CRB 2, *Rhizobium* +PGPR strain CRB 3 and *Rhizobium* + PGPR strain PUK 46B6 were 26.95, 15.02 and 14.57%, respectively, as against the uninoculated control .

***Key words :*** Co-inoculation, nodulation, PGPR, *Rhizobium*, urdbean

Being an important *Kharif* legume, urdbean [*Vigma mungo* (L.) Hepper] fixes the atmospheric nitrogen and improves the soil fertility. *Rhizobium* inoculation to the legumes not only increases the yield but also shows many beneficial effects. Sometimes indigenous rhizobial population may not be able to form effective symbiosis in field conditions due to strain competetion between introduced and native rhizobia (Pareek *et al*., 2002). Use of plant growth promoting rhizobacteria (PGPR) is often associated with increased rates of plant growth, development and yield .Further, co-inculation with *Rhizobium* and PGPR is even more effective for improving nodulation and growth of legumes (Goel *et al*.,2001; Zahir *et al*., 2004). Keeping this in view, the present study was taken up to study the co-inoculation effect of PGPRs on urd– *Rhizobium* symbiosis.

## MATERIALS AND METHODS

A three-year field trial was conducted at the Pulses and Oilseeds Research Sub-station, Beldanga, Murshidabad, West Bengal during *kharif* season of 2004, 2005 and 2006. The soil of the experimental site was sandy loam having pH 7.8, organic carbon 0.28%, available $P_2O_5$ 58 kg ha$^{-1}$ and available $K_2O$ 97 kg ha$^{-1}$.The crop variety Sarada (WBU 108) was sown on 26$^{th}$, 30$^{th}$ and 25$^{th}$ day of August during 2004, 2005 and 2006, respectively. The experiment was laid out in RBD with four replications. Besides an uninoculated control, there were seven treatments of seed inoculation with *Rhizobium* strain CRU 7 (*i.e.* Rh.), Rh. + PGPR strain CRB 1, Rh. + PGPR strain CRB 2, Rh. + PGPR strain CRB 3, Rh. + PGPR strain PUK 46B6,Rh. + PGPR strain PUK 171 and Rh. + PGPR strain KB 133. The crop was fertilized with a uniform basal dose of N : $P_2O_5$: $K_2O$ at 20:40:20 kg ha$^{-1}$ applied through urea, single super phosphate and muriate of potash, respectively. Seeds were inoculated with *Rhizobium* and PGPR prior to sowing as per treatments using 60 g culture kg$^{-1}$ seed. The crop was raised following all the recommended agronomic practices and harvested on December 02, November 23 and November 30 during 2004, 2005 and 2006, respectively.

Efficient strains of both *Rhiobium* and PGPR were obtained from All India Coordinated Pulse Improvement Project (AICPIP). Those carrier based cultures were mixed with sterilized neutral charcoal in 1:2(v/v) ratio.

In order to study the nodulation in urdbean, five plants from each plot were uprooted, their roots were gently washed with water, nodules were removed and counted. The dry weight of root nodules and crop plants was recorded after drying in the hot air oven at 80°C to constant weight. Plant height was recorded at periodic interval whereas observations were made on dry matter accumulation (DMA) at 45 DAS (days after sowing) and harvest. Seed yield in kg ha$^{-1}$ and its attributes were also recorded after crop harvest.

## RESULTS AND DISCUSSION

### Effect on crop growth

*Rhizobium* (CRU 7) alone increased the dry matter accumulation of crop plants (3.41-6.21 g plant$^{-1}$)as compared to the uninoculated control (3.38-5.85 g plant$^{-1}$) at different growth stages.These results corroborated with the findings of Prasad *et* *al.* (2002). Co-inoculation of *Rhizobium* and PGPR (CRB 2) recorded the highest mean values of DMA, being 4.24 and 7.56 g plant$^{-1}$ at 45 DAS and harvest, respectively. There were 1.02-1.24 and 1.00-1.22 fold increase in DMA due to co-inoculation with *Rhizobium* + PGPR as compared with only *Rhizobium* inoculation at 45 DAS and harvest, respectively. Irrespective of strains, co-inoculation with PGPR always recorded more plant height in comparison to the uninoculated control both at 30 and 60 DAS (Table 1). Increase in dry matter production of co-inoculated plants might be attributed to earlier and enhanced nodulation, higher $N_2$-fixation rates and a general improvement in root development (Okon and Itzigsohn, 1995).

**Effect on nodulation**

Seed inoculation with *Rhizobium* strain CRU 7 alone increased the number and dry weight of nodules as compared to uninoculated control. Co-inoculation with PGPR (CRB 2) gave the highest mean nodule number (27.76 nos. plant$^{-1}$) and weight (20.58 mg plant$^{-1}$) followed by *Rhizobium* + PGPR strain PUK46B6 (24.5 nos. of nodules weighing 17.78 mg plant$^{-1}$). Co-inoculation with PGPR at all the treatments increased the mean nodule number and weight at 45 DAS in comparison to the inoculation with *Rhizobium* alone (Table 2). It indicated that PGPR favoured the *Rhizobium* inoculum to form more nodules either by favouring its survival in the rhizosphere or by synthesis of plant growth promoting substances in developing more root hairs leading to more infection (Yahlom *et al*., 1988). The combined inoculation of PGPR and *Rhizobium* could remarkably increase the mean nodule number by 1.04 to 1.41 times and dry weight by 1.02 to 1.36 times over *Rhizobium* inoculation alone ( 19.71 nos. weighing 15.13 mg plant$^{-1}$). It was already informed that the better and effective nodulation might have resulted in better nitrogen fixation and growth of legumes (Pareek *et al*., 2002).

**Effect on seed yield**

*Rhizobium* inoculum alone increased the mean seed yield by 5.93% over uninoculated control (972.33 kg ha$^{-1}$).Dual inoculation with *Rhizobium* and PGPR strains excepting KB 133 recorded more seed yield over single inoculation with *Rhizobium*. These results were in agreement with earlier works of Prasad *et al.* (2002). Significantly highest mean seed yield was obtained due to co-inoculation with *Rhizobium* + PGPR strain CRB 2 (1234.33 kg ha$^{-1}$), followed by *Rhizobium* +PGPR strain CRB 3 (1118.33 kg ha$^{-1}$) and *Rhizobium* + PGPR strain PUK 46B6 (1114 kg ha$^{-1}$). Compared with uninoculated control, yield advantages under these treatments were 26.95, 15.02 and 14.57 %, respectively (Table 3). Such increase in seed yield might have been attributed to better crop growth (plant height of 51.42-56.77 cm and DMA of 6.45-7.56 g plant$^{-1}$ at harvest), nodulation (21.57-27.76 nos. of nodules weighing 16.21-20.58 mg plant$^{-1}$ at 45 DAS ) and improvement in yield attributes. These results corroborated with the report of Prasad *et al.* (2002) and Zahir *et al*.(2004). The beneficial effect of PGPR on *Rhizobium* had probably induced to the synthesis of growth promoting substances which could stimulate the root growth and elongation, thereby bringing about more nodulation, nitrogen fixation and crop yield (Rautela *et al.*, 2001). The increase in

**Table 1. Effect of co-inoculation with *Rhizobium* and PGPR on crop growth in urdbean**

| Treatments | Plant height (cm) | | | | | | | | | Dry matter accumulation (g plant$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 30 DAS | | | 60 DAS | | | Harvest | | | 45 DAS | | | Harvest | | |
| | 2004 | 2005 | 2006 | 2004 | 2005 | 2006 | 2004 | 2005 | 2006 | 2004 | 2005 | 2006 | 2004 | 2005 | 2006 |
| Control | 33.96 | 30.73 | 26.54 | 38.80 | 40.75 | 28.57 | 49.50 | 45.63 | 40.80 | 3.11 | 3.60 | 3.43 | 5.91 | 7.50 | 4.13 |
| Rh.(CRU 7) | 35.01 | 33.45 | 29.21 | 40.85 | 40.80 | 33.91 | 53.55 | 47.40 | 43.85 | 3.40 | 3.13 | 3.70 | 6.47 | 6.58 | 5.57 |
| Rh.+CRB 1 | 35.81 | 33.55 | 29.69 | 43.00 | 45.05 | 34.42 | 53.68 | 49.20 | 44.50 | 3.77 | 3.48 | 3.73 | 7.16 | 7.30 | 5.61 |
| Rh.+CRB 2 | 37.80 | 35.60 | 32.70 | 46.10 | 47.50 | 39.25 | 60.43 | 57.28 | 52.60 | 3.56 | 4.37 | 4.79 | 6.76 | 9.18 | 6.75 |
| Rh.+CRB 3 | 36.21 | 33.75 | 30.92 | 45.95 | 56.90 | 36.23 | 55.83 | 53.80 | 46.80 | 3.18 | 3.63 | 3.74 | 6.03 | 7.61 | 5.71 |
| Rh.+PUK46B6 | 37.29 | 33.85 | 25.59 | 46.38 | 46.45 | 31.94 | 57.50 | 55.60 | 41.15 | 4.74 | 3.65 | 3.54 | 8.99 | 7.66 | 4.45 |
| Rh.+PUK 171 | 34.72 | 32.40 | 28.52 | 38.83 | 41.75 | 32.15 | 53.33 | 47.20 | 42.98 | 4.15 | 3.50 | 3.56 | 7.88 | 7.35 | 5.11 |
| Rh.+KB 133 | 33.21 | 29.05 | 29.14 | 38.50 | 38.95 | 37.09 | 45.18 | 43.65 | 45.30 | 3.15 | 2.96 | 4.30 | 5.98 | 6.22 | 6.48 |
| **S.Em ±** | **0.98** | **0.44** | **0.78** | **0.51** | **0.45** | **1.25** | **0.76** | **1.20** | **1.45** | **0.25** | **0.15** | **0.18** | **0.29** | **0.45** | **0.20** |
| **CD(P=0.05)** | **1.39** | **1.29** | **1.30** | **1.49** | **1.31** | **2.60** | **2.24** | **3.51** | **3.01** | **0.72** | **0.43** | **0.53** | **0.43** | **0.66** | **0.42** |
| **CV (%)** | **5.50** | **2.70** | **5.80** | **2.40** | **2.00** | **7.00** | **2.90** | **4.80** | **6.00** | **13.50** | **8.20** | **9.40** | **6.00** | **9.00** | **7.00** |

DAS: Days after sowing

**Table 2. Effect of co-inoculation with *Rhizobium* and PGPR on nodulation at 45 DAS in urdbean**

| | Nodule number plant$^{-1}$ | | | Nodule weight (mg plant$^{-1}$) | | |
|---|---|---|---|---|---|---|
| **Treatments** | **2004** | **2005** | **2006** | **2004** | **2005** | **2006** |
| Control | 12.43 | 32.78 | 12.98 | 13.90 | 15.15 | 13.00 |
| Rh.(CRU 7) | 11.20 | 33.20 | 14.73 | 14.58 | 16.00 | 14.80 |
| Rh.+CRB 1 | 11.19 | 35.48 | 15.33 | 14.84 | 16.30 | 15.30 |
| Rh.+CRB 2 | 15.57 | 48.50 | 19.20 | 16.50 | 23.70 | 21.55 |
| Rh.+CRB 3 | 11.78 | 36.30 | 16.63 | 15.24 | 16.70 | 16.68 |
| Rh.+PUK46B6 | 18.25 | 41.53 | 13.73 | 17.85 | 21.00 | 14.48 |
| **S.Em±** | **0.66** | **1.37** | **0.32** | **0.64** | **0.72** | **0.50** |
| **CD(P=0.05)** | **1.92** | **4.00** | **0.95** | **1.88** | **2.10** | **1.44** |
| **CV (%)** | **9.80** | **7.60** | **4.20** | **8.50** | **8.20** | **6.10** |

**Table 3. Effect of co-inoculation with *Rhizobium* and PGPR on seed yield and its attributes in urdbean**

| | Branches plant$^{-1}$ | | | Pods plant$^{-1}$ | | | Seeds pod$^{-1}$ | | | 100-seed weight (g) | | | Seed yield (Kg ha$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatments** | **2004** | **2005** | **2006** | **2004** | **2005** | **2006** | **2004** | **2005** | **2006** | **2004** | **2005** | **2006** | **2004** | **2005** | **2006** |
| Control | 2.78 | 2.20 | 3.78 | 11.80 | 15.30 | 13.35 | 4.78 | 5.85 | 4.52 | 3.65 | 3.79 | 3.50 | 1033 | 1094 | 790 |
| Rh.(CRU 7) | 3.02 | 2.80 | 4.48 | 12.45 | 20.43 | 16.25 | 4.95 | 6.35 | 5.01 | 3.75 | 3.85 | 3.68 | 994 | 1198 | 898 |
| Rh.+CRB 1 | 3.13 | 3.18 | 4.83 | 14.18 | 17.28 | 17.10 | 5.15 | 6.20 | 5.06 | 3.75 | 3.99 | 3.68 | 1030 | 1240 | 929 |
| Rh.+CRB 2 | 3.55 | 3.60 | 5.65 | 17.31 | 21.88 | 20.50 | 5.18 | 6.45 | 5.31 | 4.10 | 3.88 | 3.79 | 1101 | 1594 | 1008 |
| Rh.+CRB 3 | 3.18 | 3.40 | 4.93 | 13.90 | 16.65 | 17.80 | 5.10 | 6.30 | 5.10 | 3.90 | 3.70 | 3.72 | 1005 | 1377 | 973 |
| Rh.+PUK46B6 | 3.31 | 3.55 | 4.20 | 17.13 | 18.70 | 13.93 | 5.20 | 6.40 | 4.64 | 3.88 | 3.77 | 3.59 | 921 | 1531 | 890 |
| Rh.+PUK 171 | 2.98 | 2.60 | 4.25 | 11.71 | 12.48 | 14.80 | 4.93 | 6.20 | 4.69 | 3.80 | 3.84 | 3.65 | 1001 | 1135 | 890 |
| Rh.+KB 133 | 2.68 | 2.30 | 5.33 | 9.36 | 11.95 | 19.35 | 4.83 | 5.80 | 5.22 | 3.70 | 3.67 | 3.76 | 1074 | 1048 | 994 |
| **SEm±** | **0.20** | **0.11** | **0.20** | **0.40** | **0.59** | **0.84** | **0.10** | **0.15** | **0.14** | **0.11** | **0.21** | **0.14** | **47.00** | **40.00** | **67.00** |
| **CD (p=0.05)** | **0.59** | **0.31** | **0.41** | **0.82** | **1.23** | **1.73** | **0.30** | **0.42** | **0.29** | **0.31** | **0.31** | **0.28** | **137.00** | **118.00** | **183.00** |
| **CV (%)** | **13.20** | **7.20** | **8.00** | **6.00** | **7.00** | **10.00** | **4.10** | **4.70** | **6.00** | **5.60** | **8.00** | **7.00** | **9.10** | **6.30** | **13.60** |

seed yield due to PGPR inoculation might also have been attributed to antagonistic interaction with various soil-borne pathogens, production and release of secondary metabolites for plant growth, or increased uptake of certain nutrients from the root environment (Jalali and Chand, 1991; Zahir *et al.*, 2004).

The study suggested that though the PGPR had favourable effect on urdbean-*Rhizobium* symbiosis , selection of effective strains which were more compatible to *Rhizobium* would be necessary for obtaining the meaningful benefits from co-inoculation. However, further studies need to be made for confirmation of the present findings at other locations under different soils and agroecological situations towards reaching a valid conclusion.

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