

Short Communication

**Nodulation and survival of acid tolerant mungbean rhizobia in acid soils**

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Mungbean [*Vigna radiata* (L.) Wilczek] is the major pulse crop of Bihar. The cultivation of mungbean with *Rhizobium* culture is recommended to ensure adequate nodulation and nitrogen fixation for maximum growth and yield. Acidic soils of plateau region are detrimental to beneficial plant-microbe interactions resulting in poor crop productivity. Nutrient constraints as a result of leaching of Ca<sup>++</sup> and Mg<sup>++</sup> have been identified as major factors resulting in poor survival, establishment and N<sub>2</sub>-fixation by *Rhizobium* sp. along with growth of host plants (4, 6). No information is available on acid tolerant mungbean rhizobia in acid soils of Jharkhand. An attempt was made to identify strains of *Rhizobium* which can grow in acid soils and fix nitrogen in mungbean.

Forty six *Bradyrhizobium* isolates were isolated from nodules of mungbean crop collected from Ranchi, Dumka and Singhbhum districts of Jharkhand. The isolates were maintained on Yeast Extract Mannitol Agar slants by regular subculturing. These isolates were subjected to physiological tests for their purity and were tested for their infectiveness and effectiveness by Leonard Jar Technique (8).

All mungbean *Bradyrhizobium* isolates were screened for their ability to grow in low pH, low P and high Al concentration in liquid basal medium imposing stress in different combinations (4). Acid tolerant mungbean isolates (BRM 1 and BDKM 4) were used for incorporation of antibiotic (str<sup>r</sup>) as per procedure described by Schwingamer and Dudman (7). The streptomycin resistance (str<sup>r</sup>) acid related stress tolerant mungbean isolates were evaluated in pot culture experiment under green house condition for survivability and nodulating ability according to the procedure of Pijnenborg *et al.* (5).

Two bulk soil samples having different pH (4.6 and 5.5) were collected from upland and medium land field at BAU Research Farm, Kanke, Ranchi for pot experiment (2001). The 4.6 pH soil sample was sandy loam having organic carbon of 4.2 g kg<sup>-1</sup>, available N 280 kg ha<sup>-1</sup>, P 4.1 kg ha<sup>-1</sup>, K 157 kg ha<sup>-1</sup>, exchangeable Al 0.31 c mol (p) kg<sup>-1</sup> soil, DTPA-extractable Zn, Cu, Mn and Fe contents as 0.38, 1.39, 6.85 and 10.84 mg kg<sup>-1</sup>, respectively and while the other soil sample (pH 5.5) was also sandy loam having organic carbon of 4.0 g kg<sup>-1</sup>, available N 290 kg ha<sup>-1</sup>, P 4.5 kg ha<sup>-1</sup>, K 160 kg ha<sup>-1</sup>, exchangeable Al 0.26 c mol (p) kg<sup>-1</sup> soil, DTPA-extractable Zn, Cu, Mn and Fe contents as 0.90, 2.73, 11.24 and 17.28 mg kg<sup>-1</sup>, respectively.

Effectiveness in terms of nodulation and dry matter yield of mungbean isolates (S<sub>0</sub> = Control, S<sub>1</sub> = BRM 1 and S<sub>2</sub> = BDKM 4) was evaluated in two soils having pH 4.6 and 5.5 in a complete randomized design alongwith four replications during the year 2001. Twenty four pots (one kg capacity) containing one kg unsterilized soil with pH 4.6 (12 pots) and 5.5 (12 pots) were arranged as per the treatments. Basal doses of fertilizer (20 kg N ha<sup>-1</sup>, 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) were properly mixed with the soil, nitrogen and phosphate were added as urea and single super phosphate, respectively. Acid tolerant mungbean (str<sup>r</sup>) were applied as broth culture (3 ml hole<sup>-1</sup> in 3 holes 4 cm deep) containing x 10<sup>8</sup> cfu ml<sup>-1</sup> followed by sowing of one seed to test isolates in each hole. The holes were covered properly with soil. The pots were irrigated as and when needed with tap water to maintain a uniform level (40% of WHC of soil) in each pot for one month including uninoculated control pots. The adhesion of rhizobia was determined according to the procedure of Caetano and Favelukes (1).

All nodules appearing in crown regions of three plants of a replication were tested for presence of str<sup>r</sup> mutants. Each nodule after surface sterilization was crushed in a test tube containing 0.5 mL sterilized distilled water. Suspension of each nodule was streaked separately in duplicate plates on solidified CRYEMA containing 500 µg mL<sup>-1</sup> streptomycin and incubated at 28±2°C for 7 days. Colonies developed were taken as positive for recovery in the nodules.

Survival and colonization of the two isolates (BRM 1 and BDKM 4) of mungbean *Bradyrhizobia* tolerant to low pH incorporated with streptomycin resistance marker were estimated in two soils (pH 4.6 and 5.5) at 7 and 14 days after emergence (DAE). The results indicated (Table 1) a better survival of the inoculants over native *Bradyrhizobia* inhabitants under varying acidic (pH 4.6 and 5.5) soil environments at early (7 DAE) and prolonged period (14 DAE)

Table 1. Survival of str<sup>r</sup> acid tolerant mungbean rhizobial isolates in mungbean rhizosphere

Isolate	X 10 <sup>4</sup> cfu g <sup>-1</sup> soil (7 DAE)			X 10 <sup>4</sup> cfu g <sup>-1</sup> soil (14 DAE)		
	pH 4.6	pH 5.5	Mean	pH 4.6	pH 5.5	Mean
BRM 1	14.5	22.2	18.3	10.5	13.2	11.8
BDKM 4	17.2	24.5	20.8	13.0	15.0	14.0
Mean	15.8	23.3	-	11.7	14.1	-
*Uninoculated control (x 10 <sup>2</sup> )	5.5	7.2	6.3	4.5	7.0	5.7

Initial inoculum load 11 x 10<sup>7</sup> cells mL<sup>-1</sup> (3 mL in one hole pot<sup>-1</sup>)

\*Estimated by MPN Technique.

of residence. Mean viable counts of inoculants reflected values of  $15.8$  and  $23.3 \times 10^4$  cfu  $g^{-1}$  at pH 4.6 and 5.5, respectively as against  $5.5$  and  $7.2 \times 10^2$  cfu  $g^{-1}$ , respectively in case of native *Bradyrhizobia* at 7 DAE. Though, population of both inoculated as well as native *Bradyrhizobia* declined over the period (14 DAE), the former maintained numerical superiority over the native counterpart. In general, soils of moderate acidity (pH 5.5) supported more population at both early and prolonged periods than soil with high acidity. Such observations have also been reported by Munns *et al.* (4). As regards relative survival of the two isolates, BDKM 4 was identified as possessing better adaptability than BRM 1 in both the acidic conditions. The former attained population of  $17.2$  and  $24.5 \times 10^4$  cfu  $g^{-1}$  under pH 4.6 and pH 5.5 acidic conditions, respectively as compared to  $14.5$  and  $22.2 \times 10^4$  cfu  $g^{-1}$  at pH 4.6 and pH 5.5, respectively by BRM 1 at 7 DAE. Similarly, an increase in BDKM 4 viable counts was noted at 14 DAE under both situations than BRM 1. These observations are in close agreement with Hartel and Alexander (3).

Results with regard to adhesion of *Bradyrhizobia* cells of the isolates of mungbean on roots of their respective host at one week of plant emergence have been depicted in Table 2. Higher adhesion of cells of all isolates alongwith respective uninoculated control on homologous host was observed in moderately acidic soil (pH 5.5) than in soil of high acidic value (pH 4.6). It ranged from the lowest of 9.2 cells root<sup>-1</sup> of mungbean plants raised in absence of isolate to the highest of 20.2 cells root<sup>-1</sup> with isolate BDKM 4 in soil of pH 5.5, maintaining significant difference among the treatments. Variation in adhesion was also significant due to use of isolates. Highest number of adhered cells on respective host was found in case of isolate BDKM 4 of mungbean (mean 18.7 cells) which were significantly superior to BRM 1 isolate and

**Table 2. Adhesion of mungbean rhizobia on homologous host root**

Isolate	Cells adhered (per root) (7 DAE)		
	pH 4.6	pH 5.5	Mean
BRM 1	10.2	12.7	11.5
BDKM 4	17.2	20.2	18.7
Uninoculated control (without str <sup>r</sup> CRYEMA)	8.2	9.2	8.7
Mean	11.9	14.0	-
Source	S,Em±	CD (P=0.05)	
S	0.21	0.63	
pH	0.17	0.51	
S x pH	0.30	0.89	

over control. Interactions of isolate  $\times$  soil pH was also significant.

Isolate BDKM 4 was identified as the most effective (no. of nodules plant<sup>-1</sup> 3.95 and adhered cells 21.1%) against isolate BRM 1 (no. of nodules plant<sup>-1</sup> 3.85, adhered cells 33.5%) and native *Bradyrhizobia* (no. of nodules plant<sup>-1</sup> 1.00; adhered cells 11.5%) (Table 3). BRM 1 was found to be the most effective strain with high nodulating ability (adhered cells 31.4%) at pH 4.6 and pH 5.5 (35.5% adhered cells) as compared to BDKM 4. However, native *Bradyrhizobia* were least efficient in infecting and forming nodules. Isolate BDKM 4 also surpassed BRM 1 and native *Bradyrhizobia* in terms of synthesis of higher nodular dry biomass. The former interacted with host more efficiently to attain a mass 10.53 mg plant<sup>-1</sup> which was significantly superior to 9.71 and 6.09 mg plant<sup>-1</sup> in the presence of BRM 1 and native *Bradyrhizobia*, respectively. Significantly higher dry nodules mass was noted in soil of pH 5.5 (10.02 mg plant<sup>-1</sup>) than 7.53 mg plant<sup>-1</sup> in pH 4.6. Host plants maintained good growth attaining mean dry

**Table 3. Infective and effective ability of acid tolerant isolates of mungbean rhizobia on homologous host**

Isolate	Number of nodules plant <sup>-1</sup>			Dry wt. of nodules plant <sup>-1</sup> (mg)			Dry wt. of shoot plant <sup>-1</sup> (g)		
	pH 4.6	pH 5.5	Mean	pH 4.6	pH 5.5	Mean	pH 4.6	pH 5.5	Mean
BRM 1	3.20 (31.4)	4.50 (35.5)	3.85 (33.5)	8.20	11.22	9.71	0.580	0.640	0.610
BDKM 4	2.70 (15.7)	5.20 (25.7)	3.95 (21.1)	8.41	12.65	10.53	0.630	0.710	0.670
Mean	2.95	4.85	-	-	-	-	-	-	-
% str <sup>r</sup> clones of mean nodule	(21.5)	(29.5)							
Uninoculated (control)	1.00 (12.2)	1.00 (10.8)	1.00 (11.5)	6.00	6.18	6.09	0.390	0.480	0.435
Overall Mean	2.30	3.50	-	7.53	10.02	-	0.533	0.610	-
Source				S,Em±	CD (P=0.05)		S,Em±	CD (P=0.05)	
S				0.10	0.31		0.012	0.036	
pH				0.08	0.02		0.009	NS	
S x pH				0.14	0.43		0.017	0.059	

Figures in parentheses indicate per cent nodule formed out of total cells adhered on root.

mass to the tune of 0.610 g plant<sup>-1</sup> in soil of pH 5.5 being significantly higher than 0.533 g plant<sup>-1</sup> recorded in soil of pH 4.6. The results of these studies showed that the survival of rhizobia in acid soil was limiting (2, 3, 6).

It may be concluded from the present investigation that acid tolerant isolates (BRM 1 and BDKM 4) exhibited better survival in soil of less acidity. The isolate BRM 1 was found to be highly infective and was able to infect and form nodules on the host. Isolate BDKM 4 showed superiority over native rhizobia in terms of dry mass of nodules and shoot.

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