

ISOLATION, BIOCHEMICAL CHARACTERIZATION AND PREPARATION OF BIOFERTILIZER USING *RHIZOBIUM* STRAINS (*VIGNA MUNGO*) FOR FARMERS USE

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Abstract: A pot experiment was conducted at Sardar VallabhBhai Patel University of Agriculture & Technology, Meerut (U.P.) to evaluate the effect of *Rhizobium* as a biofertilizers on different plant parameters related to yield performance of Black gram (*Vigna mungo*) cv.urd shekhar-2 during the period from March to June 2013. The trial composed of four treatments such as T₁=control, T₂=DAP, T₃=IARI (Urd 10B) and T₄=Native strain. Irrespective of treatment differences the black gram plant as a pulse crop showed a lag phase for slow dry matter production in early growth stage that decrease upto harvest. This greater dry matter production eventually partitioned to root length, seed number, seed weight, dry pod weight, number of pods, number of nodules and microbial count. The results revealed that biofertilization perform significant improvement in plant productivity and quality. The maximum germination and increase in plant root length, seed number, seed weight, dry weight, number of pods and microbial count was increased progressively in treatments treated with *Rhizobium*.

Keywords: Isolation, biofertilizer, *Rhizobium*

INTRODUCTION

Today as there is much scarcity of food which provide sufficient nutrient for the human body, In such condition we need to grow such food crops which are high in nutrients like protein, vitamins and several other nutritional supplements needed for a human body. The main role playing such important nutrient supplement is pulses. Pulses are such food supplements which complete the protein requirement of the body.

Black gram or *Vigna mungo* is one such crop which plays a vital role in filling up the protein deficiency (S. K. Nitu, M. M. Ud-Deen *et al.*, 2009). Nitrogen based fertilizers may serve for better growth of crop plants but efforts should be oriented towards augmenting biological nitrogen fixation mediated by microorganisms. An average area of grain legumes like soybeans, beans, or peas provides sufficient protein for 1000-2000 days for one person, whereas an average area of plant materials converted to animal protein like beef and poultry provides only for 75-250 days (Burns and Hardy, 1975).

Rhizobia encompass a range of bacterial genera, including *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium*, and *Azorhizobium*, which are able to establish a symbiosis with leguminous plants. They elicit the formation of specialized organs, called nodules, on roots or stems of their hosts, in which they reduce atmospheric nitrogen and make it available to the plant. Symbiotic nitrogen fixation is an important source of nitrogen, and the various legume crops and other species often fix as much as 200 to 300 kg. nitrogen per hectare (Peoples *et al.*, 1995).

MATERIAL AND METHOD

Plant growth and nodulation

The trial was conducted at green house of Sardar Vallabh Bhai Patel University of Agriculture and Technology. Seeds of black gram were bought and were soaked in the inoculums of Rhizobial strains to germinate for 4 days. These seeds were then sowed in 32 pots. These pots were divided in four treatments viz control (Tc), Diammonium phoaphate, IARI and native. The first treatment comprises of seeds which were soaked for 4 days in water, the second treatment comprises of same seeds and the soil contains an additional fertilizer which is DAP (diammonium phosphate). This DAP was also added to rest of the pots. The seeds present in the third treatment were soaked in Rhizobial inoculums which were taken from IARI (Indian Agriculture Research Institute) and the fourth treatment consists of seeds soaked in Rhizobial inoculum which were taken from the nearby area of Sardar Vallabh Bhai Patel University of Agriculture and Technology which was termed as Native Strain.

Two seeds were sowed in each pot and were watered at regular interval of 2 days. This irrigation schedule was maintained for 83 days and after 83 days uprooting of the plant was carried out. From each trial 3 pots were randomly selected for the recording the observation on attributes like root length, seed number, seed weight, dry pod weight, number of pods, number of nodules and microbial count.

Isolation of Rhizobial strains

The roots of Blackgram were transported to the laboratory in plastic bags. The roots of plants were

thoroughly washed and nodules were severed and sterilized in 95% ethanol for 5 s and 0.1% HgCl₂ for 5 min. Each nodule was crushed and the content of the nodule was transferred onto a petri dish with yeast-extract mannitol agar (YEMA) (Vincent 1970; Somasegaran and Hoben 1985). Petri dishes were incubated at 28 °C until typical colonies of rhizobia appeared. Single colonies were marked and checked for purity by repeated streaking on YEMA medium (Vincent 1970) and verifying a single type of colony morphology, absorption of congo red (0.00125 mg kg⁻¹) and a uniform Gram-stain reaction. From the above method the native strains were isolated and the IARI strain was also restreaked for better and fresh colonies.

Soaking of seeds

For soaking of seeds the slurry was prepared. This slurry contains about 150g of charcoal which was thoroughly mixed with 200ml of YEMA broth containing the Rhizobial strain. Like this two different slurry were prepared one with the Native Rhizobial Strain and another one with the IARI Rhizobial strain. After that 5g of seeds of black gram were taken in four petri dishes. The seeds in first petri dish were soaked in tap water, the second one was also soaked in same tap water, the third one was soaked in slurry which was containing the IARI strain and the fourth one was soaked in the slurry containing the native Rhizobial strain.

Sowing of seeds

Table 1. Length of root

TREATMENT	PLANT 1	PLANT 2	AVERAGE
Tc(control)	45	40	41.3
Tc(control)	45	40	
Tc(control)	53	25	
DAP	30	19	33.5
DAP	35	25	
DAP	59	33	
IARI	30	31	33.5
IARI	33	34	
IARI	50	23	
NATIVE	44	28	30.5
NATIVE	27	28	
NATIVE	29	27	

Seed number

The pods present on each plant were removed and the seeds from each pod were removed out counted manually.

Table 2. Seed number

	Data for 2 plants per pot(Seed number)					AVERAGE	Avg(1 plant per pot)
TC	40	33	81	105	120	75.8	37.9
DAP	26	62	105	93	130	83.2	41.6
IARI	61	87	87	115	72	84.4	42.2
NATIVE	134	95	90	90	118	105.4	52.7

After 4 days when the seeds were properly germinated then the procedure of sowing was carried out.

32 Pots were taken and for each of the four treatments 8 pots were allocated. The pots were marked as Tc (control), DAP (Diammonium Phosphate), IARI and Native. 8 kg of soil which was taken from the nearby area of the Sardar Vallabh Bhai Patel University of Agriculture and Technology were added to the pots. Except the control one all the pots were treated with 400ml of DAP solution which was having a concentration of 1.2 % (w/v). After that the germinated seeds in different treatments were sowed in the already marked pots (2 seeds per pot). Then the irrigation schedule was maintained. The pots were regularly watered at interval of 2 days.

Uprooting of plants for recording observation

After 83 days of sowing uprooting was done. Randomly 5 pots from each treatment were selected for recording the observation. The characteristics which were observed were root length, seed number, seed weight, dry weight of pods, number of pods and microbial count.

Root Length

The roots of the uprooted plants were washed by dipping them in beaker filled with tap water very firmly so that no breaking of roots occur. The roots were then blot dried by keeping them on blotting paper and remained undisturbed for half an hour. After drying the root length were measured by scale.

Seed weight

The counted seeds were weighed on weighing balance and the data was recorded.

Table 3. Seed weight

	Data for 2 plants per pot(Seed weight in gm)					AVERAGE	Avg(1 plant per pot)
TC	1.26	1.07	2.97	2.57	3.25	2.224	1.112
DAP	1.16	3.54	4.06	3.972	6.11	3.7684	1.8842
IARI	2.98	3.74	4.19	4.47	3.38	3.752	1.876
NATIVE	6.36	4.61	3.82	4.2	4.92	4.782	2.391

Dry pod weight

The pods were kept for drying for 15 days. After drying the pods were weighed on a weighing balance and the data was recorded.

Table 4. Dry pod weight

	Data for 2 plants per pot(dry weight of pods in gms)					AVERAGE	Avg(1 plant per pot)
TC	2.09	1.7	5.16	4.48	5.83	3.852	1.926
DAP	1.96	5.83	7.32	6.91	9.3	6.264	3.132
IARI	5.67	6.56	6.43	8.02	5.56	6.448	3.224
NATIVE	9.63	7.86	6.26	6.61	8.55	7.782	3.891

Number of pods

The pods present in each of the uprooted plant were counted manually and the data was recorded.

Table 5. Number of pods

	Data for 2 plants per pot(number of pods)					AVERAGE	Avg(1 plant per pot)
TC	9	8	19	22	26	16.8	8.4
DAP	7	16	30	31	29	22.6	11.3
IARI	27	23	16	32	21	23.8	11.9
NATIVE	30	23	37	15	28	26.6	13.3

Microbial count

The microbial population present in the soil was estimated in which the uprooted plants were grown.

Enrichment of sample

1g of soil sample was weighed on weighing balance and was mixed in 10ml of YEMA broth. This broth was then kept for incubation at 28°C for 48 hours. After 48 hrs of incubation, the Rhizobium gets enriched. This enriched broth of Rhizobia was then subjected to serial dilution.

Serial dilution

1ml of this enriched broth was diluted in 9ml of distilled water and this dilution was termed as 10⁻¹. Now from this dilution 1ml of the sample was taken and transferred to the next tube containing 9ml of

distilled water and this dilution was termed as 10⁻² and in the same way the serial dilutions were prepared upto 10⁻⁹ dilutions.

Plating of diluted sample

The serially diluted samples were poured on YEMA plates. Two dilutions i.e. 10⁻⁸ and 10⁻⁹ were taken for assessment of microbial population. 1ml of dilution was taken with the help of pipette and poured on the YEMA plates. The sample was then spread equally on the YEMA medium with the help of L-shaped glass rod and was incubated at 28°C for 48 hours. After 48 hours, small and isolated colonies of *Rhizobium* were observed on the YEMA plates. The small and isolated colonies were counted on colony counter and the microbial population was estimated.

Table 6. Microbial count

Treatment	Dilutions		Average
	10 ⁻⁸	10 ⁻⁹	
Tc	191	152	221.7
Tc	336	291	
Tc	284	179	
Tc	226	160	
Tc	216	182	
DAP	350	328	332.1
DAP	776	636	
DAP	286	172	
DAP	137	129	
DAP	268	239	
IARI	444	421	401.7
IARI	321	287	
IARI	782	720	
IARI	348	254	
IARI	291	149	
NATIVE	490	403	435.2
NATIVE	319	288	
NATIVE	816	788	
NATIVE	337	304	
NATIVE	315	292	

RESULT AND DISCUSSION

The growth and yield parameters of black gram such as root length, seed number, seed weight, dry weight, number of pods and microbial count were significantly increased by plant growth promoting Rhizobacteria (PGPR) application in all concentrations when compared to control. Utilization of biological fertilizer increases the other nutrient absorption, also biological phosphate fertilizer can be used as a solution for increasing phosphate and micronutrient absorption in the alkaline soil (Zahir *et al.*) in maize both qualitative and quantitative characteristics were significantly increased by phosphate-solubilizing microorganisms and also increased the growth and resistance of plants in water deficit conditions (Ehteshami *et al.*, 2007). Hoshang Naserirad *et al.* and Asad Rokhzadi indicated that inoculation with biofertilizers containing *Azotobacter* and *Azospirillum* increased the plant height, leaf number per plant, fruit mean weight and yield in compare to control (without biofertilizer). In this study also we found that due to inoculation of Rhizobial strains in two treatments of IARI and Native, there is a relative decrease in root length of plants as compared to the plants treated with DAP and the non-treated control plants. This result shows that the roots of the plant get their nitrogen source in their nearby rhizosphere so there is no need that roots could grow and extend their length to find the nitrogen at the bottom. The variants treated with Native and IARI strains also showed a remarkable

increase in the seed number, seed weight, dry weight, number of nodule and microbial count also.

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