BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2011. Vol. **8**(2), p. 833-836

Performance of Local Rhizobial strain from Marathwada Region of Maharashtra state as Inoculant of Urd bean (*Vigna mungo* (L.) Hepper) with Different Carriers

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(Received: August 10, 2011; Accepted: September 17, 2011)

A locally isolated strain of *Rhizobia (UR5)* of Urd bean from Marathwada region of Maharashtra state was assessed for its efficacy to be used as an inoculant. various carriers were inoculated with this strain in the pot trials. The assessment was done by the counting the number of nodules per plant plant and increase in weight of the plant. The newly isolated strain UR5 worked better with jaggery slurry medium in Urd bean.

Key words: Local strain specific *Rhizobia UR5, Vigna mungo,* carrier.

Biological nitrogen fixation is an important aspect of nutrition in crop production throughout the world .The process saves a huge sum of money which otherwise would have to be pumped agricultural productivity to consistently sustain an increasing world population. Among various symbiotic association of biological nitrogen fixation, the *Rhizobium* legume association is the most common in both temperate and tropical climates. *Rhizobia* plays a great role in nature as they are greatest N_2 fixers, particularly in leguminous plants and leguminous plants are the predominant source of supplying protein to the population of developing countries like India[1,2].

Looking to the need of location specific strain of *Rhizobia* to increase yield of pulses and other leguminous plants the investigations were concentrated on isolation and identifying these local strains for effective nodulation[3]. The performance of these locally isolated strains of *Rhizobia* depends up on soil environment, competitor microbes and other rhizospheric factors. In the present study most efficient local strain of *Rhizobia* (UR5) from Marathwada region of Maharashtra state was assessed for its nodulation capacity per plant, Urd bean is cultivated on a large scale now a days in this region and carrier based inoculants are widely used in the recent times. However, various factors on efficacy of this strain are already studied[4]. This paper describes the performance of the locally isolated strain of *Rhizobium* with different carriers on Urd bean nodulation and increase in dry weight.

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MATERIAL AND METHODS

Isolation and maintenance of Rhizobium

The Urd bean seeds cv.AU4 were collected from local market. The strain *RhizobiumUR5* isolated from Chudawa, Parbhani Dist .of Maharashtra state (5) of Marathwada. *Rhizobia* were isolated and maintained on YEMA medium either by sub-culturing at frequent intervals or as lyophilized cultured kept at 5 °C this system maximizes the storage period and minimizes genetic variations and contamination. The culture was also dried on porcelain beads over a desiccant in screw cap bottles. The authenticity check was carried out regularly. Their effectiveness on nodulation capacity and dry matter of Urd bean was tested. In earlier studies, strain *Rhizobium* UR5 from Chudawa village of Dist. Parbhani, Maharashtra, India was found very much effective .Their capacity to nodulate along with some carrier such as lime pelleting, Jaggery, lignite, sawdust, sterilized cow dung powder was tested by usual methods(3). The number of nodules and dry matter of plant along with different inoculants were evaluated in a pot culture experiment for three years i.e. 2008-2011.

Screening and culturing of Rhizobium

i) CRYEMA Test: 2.5 ml. of Congo red dye was mixed with a one litre of YEMA medium to prepare CRYEMA medium. Bacterial colonies on the YEMA medium were streaked on the CRYEMA medium and the petriplates were incubated at 28+ 2^0C for 5-7 days. *Rhizobial* cells forms white circular, entire, raised, convex colonies, The white colonies were picked up to produce *Rhizobium* inoculants.

ii) Microscopic Observation: Bacterial cells in the CRYEMA medium were stained with carbol fuschin and visualized under a compound microscope. This dye stains the B-polyhdroxybutyrate granule in the *Rhizobium*. The cells of those colonies having B-polyhdroxybutyrate granule were picked up to establish *Rhizobium* inoculants.

iii) Glucose Peptone Agar Test (GPA Test): *Rhizobial* colonies were streaked on YEMA medium and a master plate was made. colonies in the master plates were transferred to GPA medium by replica plating .Those colonies in the master plates fail to grow GPA medium belong to rhizobia .This test was confirmative test to test the purity of *Rhizobial* colonies[4].

Inoculation

The surface sterilized seeds were used for inoculation. The seeds were dried in shade and sown in earthen pots of respective treatments. These pots were watered with an interval of two days or on when required. After 15 days of sowing the thinning was done and five plants were maintained in each pot.

The observations were recorded for number of nodules and dry matter. Plants were uprooted carefully washed and number of nodules per plant were recorded, The nitrogen content was determined by Microkjaldhal's method .To identify the best method of inoculation, a pot culture experiment was conducted using different method of inoculation. The strain UR5 which was found superior was used. Medium type of soil was sterilized in autoclove at 30 lbs for two hours and used in the experiments. The Urd bean seeds were surface sterilized by treating with 0.1% mercuric chloride.

Carriers

The Urd bean seeds were inoculated using following carriers -

1. Lime pelleting : The seeds of Urdbean were inoculated with *Rhizobium* species coated with lime to form a pellets.
2. Rhizobium culture and jaggery: Seeds were dipped in the slurry of jaggery and *Rhizobia* broth and dried in shade and used for sowing.
3. Rhizobium culture alone: Broth culture of *Rhizobium* was diluted with sterile distilled water and the seeds were soaked in it. The seeds were air dried in shade and used for sowing.
4. Rhizobium culture and jaggery with saw dust/cow dung/lignite: The *Rhizobium* broth culture was grown for seven days. The cow /cow dung /lignite/saw dust was grinded to fine powder of 0.01mm size. The powder was mixed with the *Rhizobium* broth culture separately .After thorough mixing the powder was dried in shade under aseptic conditions and was used separately for inoculation.In control treatment the seeds were

soaked in sterile water, dried in shade and were sown in pots. The observations were recorded on nodule number and dry weight of whole plant on 40th day of sowing. The plants were grown for 40 days in uniform condition along with different inoculants. After 40 days the number of nodules per plants was recorded and the dry matter of plant was estimated by usual method. The experiment was done in three sets each and for three consecutive years i.e. 2008-2011,

Data were subjected to Analysis of Variance(ANOVA) depending upon experimental design following the procedure as given by Gomez and Gomez[6].

RESULTS AND DISCUSSION

The effect of the *Rhizobium* UR5 inoculation on nodulation of Urd bean by using various carrier is presented in table 1. The result indicated that the local *Rhizobium* strain UR5 performed well when jaggery was used as carrier and the number of nodules was significantly higher than other carriers used. The trend in results recorded during 2008-2009, 2009-2010, 2010-2011 was similar.

The effect of the *Rhizobium* UR5 inoculation on dry weight of Urd bean by using

Table 1. Effect of carrier by strain *Rhizobium* UR5 on nodulation of URD bean

S. No.	Method of inoculation	Number of Nodules per plants			Pooled Analysis
		2008-2009	2009-2010	2010-2011	
1	*Rhizobium* culture with Lime pelleting	19.59	18.86	17.89	18.87
2	*Rhizobium* culture with Jaggery	20.94	20.44	19.43	20.27
3	*Rhizobium* culture with lignite	15.52	14.09	14.65	14.75
4	Rhizobium culture with saw dust	12.39	13.24	12.88	12.52
5	*Rhizobium* culture with sterlized cow dung powder	11.25	11.74	14.58	12.52
6	*Rhizobium* culture with saw dust and cowdung powder	10.32	12.79	12.18	11.76
7	Control	7.78	8.21	9.84	8.61
	S E±	0.97	0.45	1.15	1.33
	CD at 5%	2.83	2.83	3.53	4.01

Table 2. Effect of carrier by strain *Rhizobium* UR5 on dry weight of Urd bean plants

S. No.	Method of inoculation	Dry weight of plants gms/plants			Pooled Analysis
		2008-2009	2009-2010	2010-2011	
1.	*Rhizobium* culture with Lime pelleting	0.30	0.39	0.45	0.38
2.	*Rhizobium* culture with Jaggery	0.35	0.53	0.34	0.40
3.	*Rhizobium* culture with lignite	0.31	0.41	0.44	0.38
4.	*Rhizobium* culture with saw dust	0.25	0.20	0.20	0.23
5.	*Rhizobium culture* with sterlized cow dung powder	0.22	0.20	0.18	0.20
6.	*Rhizobium* culture with saw dust and cowdung powder	0.32	0.30	0.25	0.29
7.	Control	0.23	0.23	0.18	0.21
	S E±	0.02	0.01	0.02	0.02
	CD at 5%	0.08	0.04	0.07	0.06

various carrier is presented in table 2. The result showed that the local *Rhizobium* strain UR5 performed well when jaggery was used as carrier. The dry weight recorded was significantly higher than other carriers used. The trend in results recorded during three consecutive years was similar.

Thus the results showed that this local strain seems to be very effective in Urd bean when it is used for inoculation along with jaggery. The process of infection of plants by *Rhizobia* is very much competitive[7]. The jaggery slurry method was more successful for inoculation, because in some plants the invasion of bacteria requires synthesis of important exo-polysaccharides (Marketoni.et.al., 2002) .Further investigation on local strains of bacteria and determining biodiversity of microbes is essential so that *Rhizobia* can be effectively utilized as Bio-fertilizers.

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