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Bio potential of local *Rhizobium* and its efficacy on Dry weight and Nitrogen content of Urid bean

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Abstract
The genetic diversity of *Rhizobium* is also based on geographic location on the organism which can influence on nodulation capacity of the species, having further effect on other parameters like dry weight and Nitrogen content of the plants. In the present work, some location specific strains of Rhizobium were isolated and were tested to find out their effect on dry matter and nitrogen content of Urid bean (Vigna mungo (L.) Hepper plant from the Marathwada region of Maharashtra state. The strain UR5 from Parbhani district seems to be a promising strain in increasing the dry matter and nitrogen content of the plant of Urid bean (Vigna mungo (L.) Hepper.

Keywords: *Rhizobium*, marathwada, dry matter, nitrogen content, urid bean

Introduction
Rhizobium is a gram-negative bacterium which inhabits the root nodules of most leguminous crops. These are soil bacteria that fix N2 (diazotroph) after becoming established inside root nodules of legumes. (F.G.P. Lhuissier *et al*. 2001) [4]. There are several different genera of *Rhizobium,* all of them belong to the Rhizobiales, a probably-monophyletic group of proteobacteria having unique ability to infect root hairs and form root nodules. (V.N. Matiru and F.D. Dakora 2004) [7, 12]. These are rod shaped,aerobic and motile having different species such as Rhizobium, Mesorhizobium, Bradyrhizobium, Azorhizobium, Allorhizobium and Sinorhizobium and form symbiotic relationships with legumes by responding chemotactically to flavonoid molecules released as signals by the legume host. (Shefali Poonia 2011) [10]. Urid bean (Vigna mungo (L.) Hepper. is cultivated in Maharashtra along with some other pulses such as soybean and mung on a large scale, Rhizobium plays an important role in Nitrogen fixing particularly in leguminous plants, The Urid bean (Vigna mungo (L.) Hepper. Which becomes a popular source of protein for the developing countries like India (Rhandwa 2003) *et al*. [9]. These plant compounds induce the expression of nodulation (nod) genes in Rhizobium, which in turn produce lipo-chitooligiosaccharide (LCO) signals that trigger mitotic cell division in roots, leading to nodule formation (Matiru and Dakora, 2004; Dakora, 1995; Lhuissier *et al.,* 2001) [7, 12, 2, 4]. The location specific *Rhizobium* are having an inherent genetical capacity which can cause an increase in nodulation capacity of legume selection of these efficient strain will not only increases the nodulation capacity of these nitrogen fixing species but also it will ultimately have an effect on other parameters of the plants like dry matter and nitrogen content of the plants.

Material and methods
The root nodules were collected from different locations from different three districts of Marathwada region of Maharashtra, in kharif season in 2000-2002. Table no. 1.
Isolation and maintenance of *Rhizobium*, the collected roots were thorolly washed under tap water to remove adhering soil particles. Pink colored nodule was selected for isolation of *Rhizobium* bacteria these nodules were crushed with the help of sterile forceps and the 100 μL contents were spread on Nutrient Agar (NA) plate. Nodules were safely cut from the root and were washed under running tap water and then for 30 sec in 70% ethanol solution. They were then treated with 0.1% $HgCl_2$ for 2 min and successively washed three times with sterile distilled water under aseptic condition for 1 min each. Geetha Rajendran 2012 [6]. *Rhizobium* were isolated and maintained on YEMA medium either by sub-culturing at the strain *Rhizobium* UR5 isolated from Chudawa, Parbhani Dist. of Maharashtra frequent intervals or as lyophilized cultured kept at 5 0 C this system maximizes genetic variation and contamination. The culture was also dried on proclaim beads over a desiccant in screw cap bottles. In earlier studies, strain Rhizobium UR5 from Chudawa village of Parbhani district of Marathwada,

these strains were tested for their capacity about enhancement of dry matter and Nitrogen content of the Urid bean (Vigna mungo (L.) Hepper plant. The Urid (Vigna mungo (L.) Hepper. bean seeds c.v.AU4 were collected from local market of Nanded.

Screening and culturing of Rhizobium

1) CRYEMA Test: 2.5 ml of Congo red dye was mixed with a one liter of YEMA medium to prepare CRYMA medium. Bacterial colonies on the YEMA medium were streaked on the CRYMA medium. Bacterial colonies on the YEMA medium were streaked on the CRYMA medium and the Petri plates were incubated at 28+2 0 C for 5-7 days. Rhizobium cells from white circular, entire convex colonies, the white colonies were picked up to produce *Rhizobium* inoculants.
2) Microscopic observation: Bacterial cells in the CRYMA medium were stained with carbol fuchsin and visualized under a compound microscope. This dye strains the B polyhydroxybutrate granules were picked up to establish Rhizobium inoculants.
3) Glucose peptone Agar test (GPA Test): Rhizobium colonies were streaked on YEMA medium and a master plate was made, colonies in the master plates were transferred to GPA medium by replacing plating. Those colonies in the master plates fail to grow GPA medium belong to Rhizobia, This test was confirmative test for the purity of *Rhizobium* colonies.

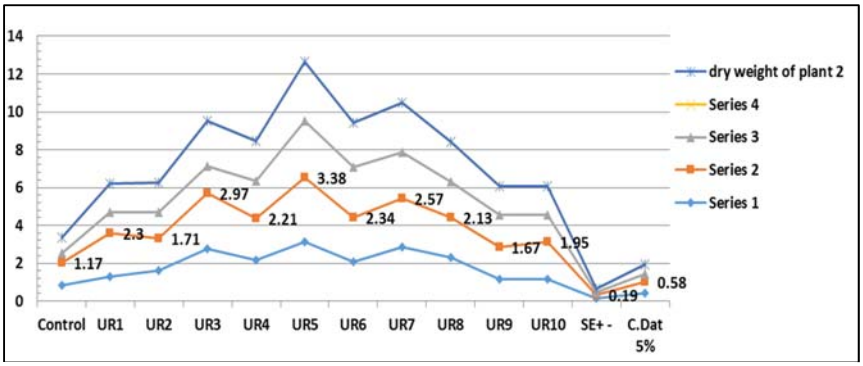
Inoculation the surface sterilized seeds were used for inoculation. The seeds were dried in shade and sown in earthen pots of respective treatments. These pots were watered with an interval of two days or on when required. After 15 days of sowing the thinning was done and five plants were maintained in each pot. The observations were recorded and plants were uprooted carefully washed and number of nodules per plant was recorded. The nitrogen content was determined by Microjadhals method, to identify the best method of inoculants, 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 autoclave at 30lbs for two hours and used in the experiments.

Table1: Location wise isolates of Rhizobium obtained from Urid bean (Vigna mungo (L.) Hepper.

Sr. No	Isolates	Location	District
1	UR1	Degaon	Nanded
2	UR2	Dhamdari	Nanded
3	UR3	Dour	Nanded
4	UR4	Kharbi	Nanded
5	UR5	Chudawa	Parbhani
6	UR6	Wanegaon	Nanded
7	UR7	Hingoli	Hingoli
8	UR8	Chitgiri	Nanded
9	UR9	Gour	Parbhani
10	UR10	Limbgaon	Nanded

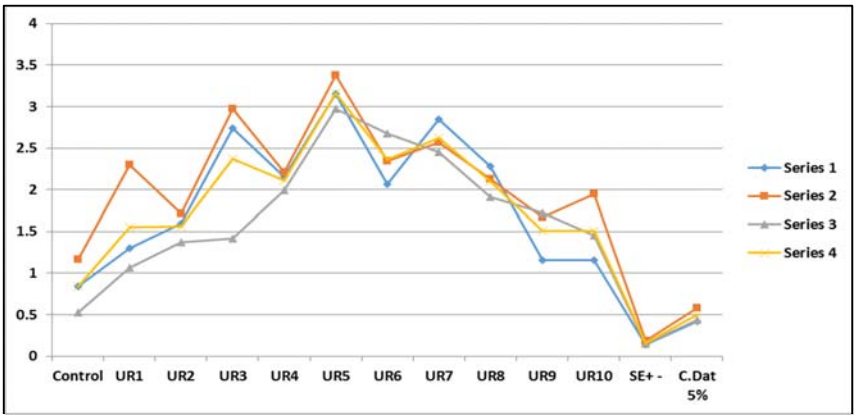
Result and discussion:

The crop pattern of the region is uniform but the genetic variability of symbiont and the host are important as it present the gene sequencing of host as well as symbiont is being carried out in order to have optimum Nitrogen fixation (GiIbert et.al 2001) [5]. The results clearly indicate that the strain UR5 which has capacity of produced height amount of Nitrogen content and dry matter in Urid bean (Vigna mungo (L.) Hepper plant. The overall enhancement of nitrogen content and also increase the carbohydrate metabolism of plant (Streeter. 1980) [11], Hence there is increase in Dry matter content and ultimately there is increase in Nitrogen content in plants the collection identification and investigation on biodiversity of higher plants and microorganisms still continuous process (Balmford et.al.2005) [1] however studies on biodiversity of location specific Rhizobia with particular effect is useful in these directions of diversity investigations and the practical utility of biodiversity of Rhizobium.



Y-axis % Nitrogen content X-axis- Isolate Number

Fig 1: Effect of different isolates of *Rhizobium* on dry weight of Urid bean (*Vigna mungo* (L.)



Y-axis % Nitrogen content X-axis- Isolate Number

Fig 2: Effect of different isolates of *Rhizobium* on % Nitrogen content of Urid bean (Vigna mungo (L.) Heppers Y-axis % Nitrogen content X-axis- Isolate Number

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