RESEARCH ARTICLE

Biodiversity of *Rhizobium* from Marathwada region.

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ABSTRACT

The *Rhizobium* plays a great role in fixation of nitrogen with the help of leguminous plants by symbiotic association. The present paper gives an idea of the Biodiversity of *Rhizobium* species from Marathwada region of Maharashtra state. The study is important as Marathwada region is an area with uncertain range and drought. The study includes identification of *Rhizobium* strains only,further investigation of identification of species is under way.

Keywords: Boidiversity, Nitrogen fixation, *Rhizobium strain*, Marathwada,

INTRODUCTION

The biodiversity refers to the variety of world biological sources living organism, population, community, ecosystem, landscape and biosphere levels. It also refers to all type of variation among living organisms and the ecosystem complexes in which they occurs. It is a function not simply of the number of the ecosystem and animal species is existence at any time but also of genetic differences with individual species. This great diversity of the worlds plant microbes and animal species has intrinsic value to human existence and commerce (Groombridge, 1992). The actual number of existing species may be particularly micro-organisms such as Bacteria and viruses out of 15 million species only 7% have been identified Sahu (2004).

The agricultural biodiversity has become recent accept were the new genetic verities of crop plants are being searched and identified, legumes are a popular matter of investigation the productivity of the legumes is dependent up on the strain of *Rhizobium* species associated with them (Rhandwa et.al 2003) IBP world catalogue of *Rhizobium* listed about 3000 *Rhizobium* strains from legume in different countries(Gangavane, 2000). *Rhizobium* which belongs to the Rhizobiaceae family (Deshwal and Chubey 2014) has a beneficial effect on growth of plants (Shahzad et al., 2012) *Rhizobium* species can exist as free living nitrogen fixing endosymbionts of leguminous plants associated with plant roots.(Gothwal et al., 2008); (Shamseldin et al., 2008) .

Biodiversity of these *Rhizobia* strains is a matter of investigation generally no systematic study on this aspects particularly the geographical variation in the strains and the nitrogen fixation capacity of these strain is not seriously consider (Balmford et al., 2005). The present paper describes biodiversity of *Rhizobia* associated with legumes from Marathwada region. These legumes are Urid bean (*Vigna mungo* L) Hepper Soybean (*Glycine max L*) which has been introduce in the area. The microbial diversity of *Rhizobia* strains from 10 different locations of Marathwada region of Maharashtra state is depicted in the present paper (Table 1).

MATERIAL AND METHODS:

The *Rhizobial* strain were collected from various locations from three districts in Marathwada i. e. Nanded, Hingoli, and Parbhani. from two cultivars of Urid bean (*Vigna mungo* (L.) Hepper and Soybean (*Glycine max L*) seeds of these plants were collected from local market of Nanded. The different parameters of infection in the root of leguminous plants and ability to have their effect are determined by different parameters however these *Rhizobial* strains could not be identified on specific level of species and variety. The names temporary given to these strains are to be verified and work is going on these accept. Table 1.

Isolation of *Rhizobium* *:*

The root nodules of Urid bean (*Vigna mungo*(L.) Hepper and Soybean (*Glycine max L*) were collected from different locations, the colour of nodules are white, brown, pink and green ,depending on the pigment in them for isolation of *Rhizobium* pink colour nodules was selected (Vincent 1970). These nodules were washed in tap water to remove the adhering soil particles from nodules. The nodules were surface sterilized with 70% ethanol or 0.1% mercuric chloride and wash with sterile distilled water. These nodules crushed with saline solution and identified with Cryma test, Glucose peptone test, Hoofers alkaline broth test and lactose agar.

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 petriplates were incubated at 28+2^0 C for 5-7 days. *Rhizobial* cells from white circular, entire convex colonies, the white colonies were picked up to produce *Rhizobium* inoculants (Singh et al., 2008).

2) Microscopy:

Under a phase contrast microscope, *Rhizobia* are discernible even a simple water mount and shows presence of B-hydroxybutyrate with in the cell. (Pelezar et al., 1977). The *Rhizobium* cells has a large irregularly shaped nuclear region in the centre surrounded by a narrow region of denser of protoplasm (Mosse, 1964).

3) 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 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 *Rhizobial* colonies.

4) Hofer s alkaline broath test

The test is based on the fact that *Agrobacteria* grow at high PH level, while *Rhizobia* are not grow on high PH.

5) Lactose Agar

Agrobacterium utilized lactose by the action of the enzyme ketolactose were as *Rhizobia* cannot utilize the sugar this can be detected on agar medium containing lactose 10gm/L. (Rao, 1971) all these tests were carried out with three replicates.

Inoculation

Nitrogen is one of the nutrient required in highest quantity to soybean and Uridbean these legume conduct biological Nitrogen fixation (BNF)when associated with nitrogen fixing bacteria (Wendland et al., 2010). 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, medium type of soil was sterilized in autoclave at 30lbs for two hours and used in the experiments. The different strains isolated from different locations was tested with Urid bean (*Vigna mungo*(L.) Hepper and Soybean (*Glycine max L*) cultivars. The strain UR5and SR5 which was obtained from Parbhani district was found as compere to control.

Table 1: Location wise isolates of *Rhizobium* obtained from Urid bean (*Vigna mungo* L.) Hepperand Soybean (*Glycine max L*) .

Sr. No	Isolates	Location	District
1	UR1/SR1	Degaon	Nanded
2	UR2/SR2	Dhamdari	Nanded
3	UR3/SR3	Dour	Nanded
4	UR4/SR4	Kharbi	Nanded
5	UR5/SR5	Chudawa	Parbhani
6	UR6/SR6	Wanegaon	Nanded
7	UR7/SR7	Hingoli	Hingoli
8	UR8/SR8	Chitgiri	Nanded
9	UR9/SR9	Gour	Parbhani
10	UR10/SR10	Limbgaon	Nanded

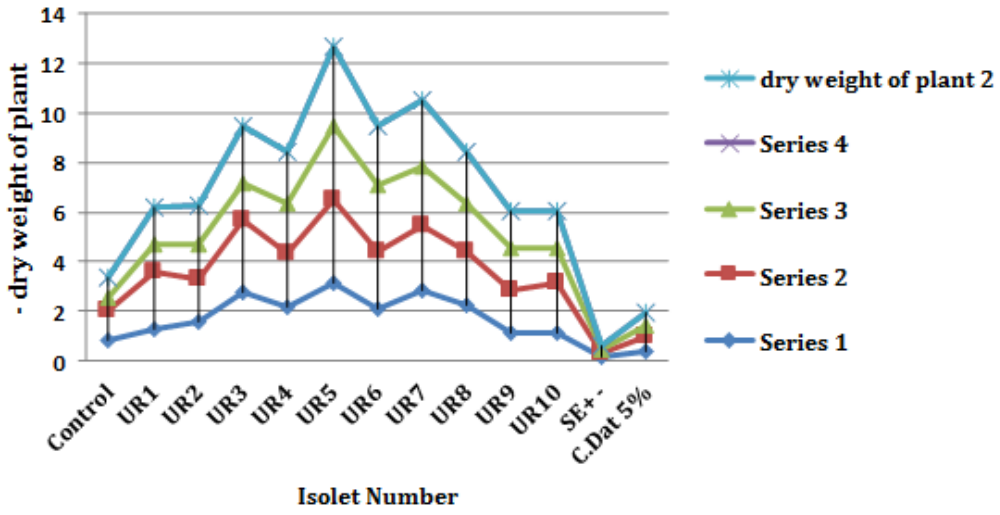

Fig .1: Effect of different isoletes of *Rhizobium* on dry weight of Urid bean (*Vigna mungo* (L.) Hepper

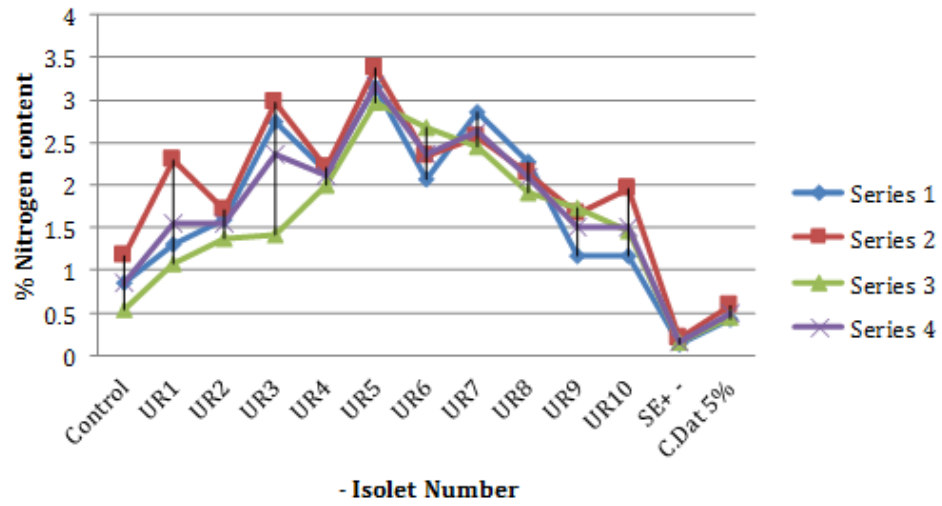

Fig.2. Effect of different isoletes of *Rhizobium* on % Nitrogen content of Urid bean(*Vigna mungo* (L.)Hepper

Table 2 : Effect of different isoletes of *Rhizobium* on Number of Nodules per plant of the Soybean (*Glycine max. L*).

Isolets No	Number of Nodules per plant			Pooled analysis
	2008-09	2009-10	2010-11	
Control	2.93	3.91	7.09	4.64
SR1	12.67	11.95	10.07	11.56
SR2	5.20	6.48	7.34	6.34
SR3	6.25	7.62	8.93	7.30
SR4	9.68	10.08	9.35	9.70
SR5	15.27	16.13	13.80	11.50
SR6	9.91	7.29	8.61	8.63
SR7	10.28	8.82	11.21	10.10
SR8	9.66	9.72	10.38	9.92
SR9	11.67	13.75	10.59	12.00
SR10	9.04	7.19	10.37	8.86
SE+-	1.13	0.61	0.71	0.85
C.D at5%	3.33	1.80	2.11	8.86

RESULTS AND DISCUSSION

The result of present investigation on *Rhizobial* Biodiversity of Marathwada region is shown in the Fig. 1 and 2. shows, the dry weight and percent nitrogen content of the plant was determined after 40 days of sowing. The dry weight is in gram per plant. The results clearly indicate that the strain UR5 which has capacity of produced high amount of percent nitrogen content and dry matter in Urid bean (*Vigna mungo* (L.) plant. The overall enhancement of nitrogen content and also increase the carbohydrate metabolism of plant (Streeter 1980). Table no 2 shows. The number of nodules were counted after 40 days of sowing the results clearly indicates that train SR5 which produced heighest number of nodules i.e 15.7, 16.13 and 13.80 in soybean (Glycine max L) plant as compared to control. These results were submitted to analysis of variance and regression test at 5% probability through Sisvar stastical programme (Siswar 2011) number of nodules, nitrogen content and dry weight of selected plants. However the technological approaches on Microbial diversity which is the least investigation matter is a subject for forever investigation

Conflicts of interest: The authors stated that no conflicts of interest.

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