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Evaluation of effective local rhizobial isolates for nitrogen fixation responsiveness variety of soybean (*Glycine max*)

R. Naveen Kumar, R. Subhash Reddy, R. Sudhkar and B.H. Sarvani

Department of Agricultural Microbiology and Bioenergy; College of Agriculture, Rajendranagar, Hyderabad 500 030, Andhra Pradesh, India

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ABSTRACT

In the present investigation, a total of fourteen rhizobial isolates were obtained from the Soybean root nodules and rhizospheric zones of different parts of Adilabad district. The bacterial isolates were authenticated as *Bradyrhizobium* on the basis of cultural, morphological, biochemical and nodulation tests. These rhizobial isolates were screened for plant growth promoting attributes such as phosphate solubilization, IAA and siderophore production. Four popular local varieties viz., JS-335, PK-1029, MACS-124 and LSB were selected and evaluated for nitrogen fixation responsiveness. Among the four varieties tested, JS-335 showed better response to commercial *Bradyrhizobium* (CRIDA Soya 809). Further, JS-335 variety was tested against the rhizobial isolates for effective root nodulation, of which SBR-8 resulted in effective symbiosis indicating, more nodule number, nodule and plant dry weight. Total Nitrogen content of plant and soil were also increased along with microbial population in the rhizosphere of soybean crop. From the findings of the research, it is concluded that, SBR-8 rhizobial isolate is better suited for the development of a commercial biofertilizers for JS-335 variety.

Key words : Bradyrhizobium, *Soybean, rhizobial isolates, Nitrogen fixing responsiveness, IAA production.*

Introduction

Soybean, (*Glycine max*) is one of the oldest crops known to man. The Soybean belongs to the family *Fabaceae* family. It is the major oil seed crop in the world, accounting for nearly 50% of the total oil seeds acreage as well as production. It is a highly nutritive energy rich legume primarily utilized as the source of protein and oil which provides approximately 60% of vegetable protein and 30% of oil to the world. Among legumes soybean is an important N_2 fixing crop, cultivated throughout the world which form root nodules and reduces atmospheric nitrogen in symbiosis with the *Bradyrhizobium japonicum* by Biological Nitrogen Fixation (BNF) mechanism. Being a legume, it is adding about 65 – 100kg N ha^{-1} to soil, helping to increase the yields of non legume crops (Fujitha *et al.* 1992).

Rhizobia a non sporulating, gram negative rod shaped soil bacteria, establish an effective symbiotic relation ship with legumes. It could reduce the nitrogenous fertilizer requirement to other crops (Beauchamp *et al.* 1996). Rhizobia, apart from symbiotic association are efficient producers of plant growth promoting substances as IAA, siderophores (Ghosh *et al.* 2008; Prabharathi *et al.* 2008). Majority of the Indian soils are devoid of an efficient *Bradyrhizobium japonicum*. The isolation of an efficient strain directly cultures from effectively nodulated soybean plants or local rhizosphere isolates are

***Corresponding author's email:** (malleshmh3670@gmail.com), [1]Zonal Agricultural Research Station, University of Agricultural Sciences, Bengaluru

easily isolatable and can be identified for their effective symbiotic associations.

Though the soybean cultivation was introduced in the Adilabad district, the crops failed to establish due to the absence of specific indigenous rhizobial strains in soils. As an alternative to the increased expensive N fertilizer, the most efficient strains of rhizobia having ability to nodulate soybean have to be isolated, purified and multiplied on mass scale, to be supplied as a suitable biofertilizers to the farming community.

An investigation was taken up to isolate and purify the native rhizobial isolates present in root nodules in rhizospheric zone of Soybean, from different localities, to evaluate their effectiveness in Symbiotic N fixation and on other growth promoting parameters.

Materials and Methods

Soil Sample Collection

Soil samples and root nodules were collected in the polythene bags as per the standard method of Jackson, 1973 from the Soybean growing areas of Adilabad District viz., Dhanor, Derapur, Muthnor, Ganrapur, Inderrelly, Utnoor Bersaipet, Salewada, Ponnam and were analysed chemically.

Isolation of bacterial isolates

Soil samples were diluted by serial dilution method as described by Vlassak (1992) and the dilutions (10^{-1} to 10^{-4}) were spread on the selective media plates i.e. Yeast Extract Mannitol Agar (YEMA) and incubated at 30°C for a period of 24-72h.

Characterization of bacterial isolates

The isolates that were likely to be *Rhizobium* colonies were picked up from the YEMA plates and proceeded for confirmation by studying the cultural, morphological and biochemical examination as given below.

Cultural Characterization: After incubation, cultures were studied for their colony characters such as size, shape, margin, consistency, pigmentation etc. as per Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994).

Morphological Characters: Purified cultures were further studied for their cell morphology viz., Cell shape, Cell arrangement, response to the gram stain and for spore formation under 100X magnification of light microscope.

Biochemical Characters: The cultures were finally confirmed by following special procedures as described in Bergey's manual of systemic bacteriology and the confirmed pure isolates were maintained on the respective slants in refrigerator at 4°C.

Biochemical tests performed and the protocols followed are briefly outlined

1. Yeast Extract Mannitol Agar with Congored test (YEMAC): The isolates were streaked on YEMAC media plates and incubated at 28±2°C for 48-72h. *Rhizobium* isolates do not absorb colour and remain white in colour.

2. Hofer's Alkaline Test: The isolates were streaked on Hofer's alkaline media plates and incubated at 28±2°C for 48-72h. *Rhizobium* does not grow on the media plates. (Vincent, 1970) whereas, the non-*Rhizobium* colonies will absorb the colour and form red colonies on media plates.

3. Indole production test: Sterilized Hydrogen sulfide- Indole-Motility agar (SIM agar) slants were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28±2°C. Following incubation, 10 drops of Kovac's indole reagent were added to each tube. The isolates showing production of red colour were recorded as positive for indole production.

4. Methyl Red Test: Sterilized glucose- phosphate broth tubes were inoculated with the test culture and incubated at 28±2°C for 48h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. Red colour production was taken as positive and yellow colour production was taken as negative for the test.

5. Voges Prausker's Test: To the presterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37°C for 48h. After incubation ten drops of Baritt's reagent A was added and gently shaken followed by addition of 10 drops of Baritt's reagent B. Development of pink colour in the broth was taken as positive for the test.

6. Keto-lactose agar test: Test isolates were streaked on the Ketolactose medium and these plates were incubated at 26°C to 28°C for 7 days. After the incubation period plates were flooded with Benedict solution in order to test for the production of Lactonic acid. The change in colour of Benedict's solution from blue to yellow around the colonies indicated

conversion of lactose to Lactonic acid. Isolates exhibiting negative reaction for the test were considered positive for *Rhizobium*.

7. Growth on Bromothymol blue medium: Freshly prepared YEMA plates containing Bromothymol blue medium having a pH of 6.8 were streaked with the *Bradyrhizobium* isolates and incubated for 2 to 3 days at room temperature. The acid and alkali production will be indicated by the change in colour into yellow and blue. Slow growing rhizobia showed an alkaline reaction in this medium turning the dye blue.

8. Growth on Glucose Peptone Agar: The method described by Vincent (1970) was followed; young cultures were streaked onto freshly prepared glucose peptone agar plates and incubated at 26°C – 28°C for 48 h or more. No growth and no change in pH of the medium was confirmatory test for *Rhizobium*.

9. Citrate Utilization: Isolates were streaked on Simmon's citrate agar slants and incubated at 28±2°C for 24h. Change in colour from green to blue indicates the positive reaction for citrate utilization.

10. Catalase test: This test was performed by adding 2-3 drops of 3% Hydrogen peroxide in fresh broth cultures of the bacterial isolates. Transfer a colony of the organism on a microscope slide and add the drop of 3% Hydrogen peroxide. If catalase is present, the Hydrogen peroxide is broken down in to water and oxygen, which result in the immediate formation of gas bubbles.

11. Nodulation test: The isolates which were confirmed as Rhizobia by the earlier described methods were used for the nodulation test. The method described by Vincent (1970) was followed.

12. Oxidase Test: The overnight cultures of the test isolates were spotted on plates poured with sterile Trypicase Soy Agar (TSA) and the plates were incubated for 24 h at 28 ± 2°C. After incubation, 2-3 drops of N, N, N', N'- tetramethyl- p-phenylenediamine dihydrochloride (Wurster's reagent) were added onto the surface of growth of each test organism. The isolates showing change of colour to maroon were noted as oxidase positive.

Screening of pure *Rhizobium* isolates Plant growth promoting attributes

Phosphate solubilization

This test was performed following spot inoculation on Pikovskaya's medium. Clear zone around the colonies indicates phosphate solubilization.

IAA production

Production of indole acetic acid (IAA) was detected as described by Brick *et al.* (1991). Bacterial isolates were grown for 72 h in yeast extract broth tubes at 36±2°C. fully grown cultures were centrifuged at 3000rpm for 30 min. the supernatant (2 mL) was mixed with two drops of Orthophosphoric acid and 4 ml of the Salkowaski reagent (50 mL, 35% of Per chloric acid, 1 mL of 0.5M $FeCl_3$ solution). Development of pink color indicates IAA production.

HCN production

HCN production was tested by the method of Lorck (1948). Yeast extract mannitol broth was amended with 4.4 g glycine/L and bacteria were streaked on modified agar plate. A Whatman filter paper no.1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed at the top of the plate. Plates were sealed with Para film and incubated at 36±2°C for 4 days. Development of orange to red color indicates HCN production.

Siderophore production

Siderophores were detected quantitatively by CAS Shuttle Assay (Schwyn and Neilands, 1987). 0.5% of cell free culture supernatant was added to 0.5% CAS (Chrome Azurol Sulphate) assay solution and absorbance was measured at 630 nm against a reference consisting of 0.5 mL uninoculated broth and 0.5 mL CAS reagent. Siderophore content in the aliquot was calculated by using the following formula:

$$\% \text{ siderophore units} = \frac{Ar - As}{Ar} \times 100$$

Where, A_r = Absorbance of reference at 630nm A_s = Absorbance of sample at 630nm.

Evaluation of local popular varieties for effective nodulation

Soil is collected from the college farm and its initial nutrient status was analysed. Soil was sterilized at 121°C & 15lb pressure for three consecutive/successive days and was used for filling the 10 kg capacity plastic pots. Each pot is filled up with 8Kg of soil. Sprouted seeds were placed in each pot with sterilized forceps and care should be taken that optimum moisture conditions were maintained throughout the experiment period. One ml of broth containing

10 rhizobia per mL was used to inoculate the soybean seedlings.

The entire pot culture evaluation was studied under two main experiments

Expt 1: Four popular varieties of soybean viz JS – 335, PK – 1029, MACS – 124 and LSB – 1 were inoculated with a commercially available biofertilizers CRIDA Soya 809: They were evaluated by nodulation tests for N_2 fixation responsiveness in a CRD design comprising of three replications and 12 treatments as T_1 (JS 335+ CRIDA Soy 809); T_2 (JS 335 – with RDF for N @ 12 kg /acre); T_3 (JS 335 – Control); T_4 – (PK 1029 + CRIDA Soya 809); T_5 (PK 1029 + RD F – N); T_6 (PK 1029 control); T_7 (Macs – 124 + CRIDA Soya 809): T_8 (Macs – 124 + RDF–N), T_9 (MACS – 124 Control T_{10} (LSB -1 +Crisa Soya 809); T_{11} (LSB + RDF – N); T_{12} (LSB – 1 Control) respectively to identify a suitable inoculate responsive soybean variety.

Expt 2: In experiment best N responsive soybean variety JS – 335 was selected for studying the Nitrogen fixing efficiency of the none authenticated local rhizobial strains in a pot culture experiment designed in CRD with 3 replications an all treatments as T_1 – SV 1 (JS 335) + SBR – 4; T_2 (SV, JS 335) + SBR -5; T_5 – JS 335 + SBR – 8; T_6 – JS 335 + SBR – 9; T_7 JS 335 + SBR – 8 JS 335 + SBR -11 ; T_9 – JS 335 + SBR -13; T_{10} JS 335 – Control; T_{11} JS 335 + CRIDA Soy 809 respectively.

Results and Discussion

Isolation of *Rhizobium* isolates

A total of fourteen bacterial colonies similar to *Rhizobium* were obtained from the rhizosphere and root nodules of Soybean crop from different parts of Adilabad district. Finally, the fourteen bacterial colonies were confirmed as *Rhizobium* sp. based on the cultural, morphological and biochemical examination. All the ten bacterial isolates were found to be small, round and form watery mucoid colonies on the YEMA plates. Under microscope, they were non-sporulating, gram -ve and rod shaped cells (Table 1). Based on the physiological studies, they were failed to grow on Hofer's alkaline medium and Keto lactose agar plates (Table 2). As per the biochemical properties are concerned, they were found to be Indole, Catalase, oxidase and VP Positive, whereas Negative for citrate utilization (Table 3). The results were found to be similar with Deka and Azad 2006, where all the *Rhizobium* isolates were positive for oxidase, catalase, citrate and urease tests and negative for indole, gelatin liquefaction tests. The results obtained were also in conformity with the studies of Bromfield and joxes (1980), Bromfield and Kumar (1983). Absence of growth in Hofers alkaline medium (Hofer, 1935), Color change in lactose agar (Bernerts and Deley 1963) are considered as the important biochemical characters aiding in the identification of *Rhizibium*.

Joseph *et al.* (2007) isolated a total of one hundred and fifty isolates from different soils of chickpea from the vicinity of Allahabad and among them thirty five isolates were identified as *Rhizobium* on the basis of morphological and biochemical characterization.

Screening of pure *Rhizobium* isolates Plant growth promoting attributes

All the bacterial isolates were screened for plant

Table 1. Cultural and Morphological characterization of *Bradyrhizobium* isolates

No. of isolates	Colony Morphology					Cell morphology		
	Size	Shape	Color	Elevation	Pigmentation	Shape	Arrangement	Gram Reaction
Fourteen	Small	Regular	Watery	Elevated	No	Rod	Single, Slender and Short rods	Gram -ve

Table 2. Physiological characterization of *Bradyrhizobium* isolates

No. of isolates	Physiological Characters			
	Growth on Glucose peptone agar plates	Growth on Hofers alkaline medium	Production of Acid/Alkali	Keto-lactose utilization
Fourteen	No growth	No growth	Alkaline	Not utilized

growth promoting attributes and the results were depicted in the Table 4.

Among the fourteen isolates tested, six isolates were found to be capable of solubilizing tricalcium phosphate. Out of ten, SBR-8 showed highest zone of 16mm with 60% Solubilization Efficiency (SE), followed by SBR-4, SBR-6 and SBR-11 showed 14mm zone with 40% S.E. Remaining isolates SBR-5, SBR-7 showed SE of 20% with 12mm of solubilization zone. Ten of the total isolates were identified as Indole Acetic acid (IAA) producers, of which SBR-8 and SBR-4 were found to be strong. Two isolates (SBR-6 and SBR-11) produced IAA in fewer amounts, while the remaining isolates SBR-5, SBR-7, SBR-9, SBR-10, SBR-13 and SBR-14 showed moderate production of IAA.

Results were in conformity with Verma *et al.*, (2010) who evaluated *Rhizobium* spp. for *in vitro* PGP properties and concluded that the bacterial strain was found to be positive for IAA and phos-

Table 3. Biochemical and Plant Growth Promoting Characteristics of *Brady rhizobium* isolates

Isolate	Biochemical and Plant growth promoting characteristics					
	Indole test	Methyl Red test	Voges-Proskaur test	Citrate utilization	Oxidase test	Catalase test
SBR-1	+	+	+	_	+	+
SBR-2	+	+	+	_	+	+
SBR-3	+	+	+	_	+	+
SBR-4	+	+	+	_	+	+
SBR-5	+	+	+	_	+	+
SBR-6	+	+	+	_	+	+
SBR-7	+	+	+	_	+	+
SBR-8	+	+	+	_	+	+
SBR-9	+	+	+	_	+	+
SBR-10	+	+	+	_	+	+
SBR-11	+	+	+	_	+	+
SBR-12	+	+	+	_	+	+
SBR-13	+	+	+	_	+	+
SBR-14	+	+	+	_	+	+

'+' indicates Positive for production '-' indicates No production

Table 4. Plant Growth Promoting Attributes of Soybean *Brady rhizobium* isolates

Isolate	IAA S.E (%)	Phosphate Solubilization	Hydrogen Cyanide production	Siderophore production	Nodulation
SBR-1	–	–	–	–	+
SBR-2	–	–	–	–	+
SBR-3	–	–	–	–	+
SBR-4	+++	40	++	20	+
SBR-5	+	20	–	–	+
SBR-6	++	40	+	–	+
SBR-7	+	20	—	–	+
SBR-8	+++	60	+++	40	+
SBR-9	+	–	—	–	+
SBR-10	+	–	—	–	+
SBR-11	++	40	+	–	+
SBR-12	–	–	—	–	+
SBR-13	+	–	—	–	+
SBR-14	+	–	—	–	+

* Solubilization Efficiency '+++' is Strong production '++' is for slightly strong production '+' indicates weak production '-' indicates No production

phate solubilization. Similarly, Joseph *et al.*, (2007) isolated thirty five *Rhizobium* spp. from the rhizosphere and screened in vitro for their plant growth promoting characteristics. Results revealed that 85.7% of *Rhizobium* isolates showed IAA production.

In case of HCN production, three isolates showed positive results, of which SBR-8 showed strong production. SBR-4 showed moderate production, while other two (SBR-6 and SBR-11) showed weak production. Out of all the fourteen isolates, only two isolates SBR-8 and SBR-4 were produced siderophores. Similarly, Chandra *et al.* (2007) isolated *Meso rhizobium loti* MP6, from root nodules of *Mimosa pudica* which induced growth and yield of *Brassica campestris* through plant growth promoting attributes. The isolate MP6 showed production of IAA, HCN, phosphate solubilization. The effectiveness of the isolates of *Rhizobium* Spp in nodulation was shown to be positively correlated with IAA Production ability (Etesami *et al.* 2008). Mahmood and Abd – Alla (2001) reported that half of the microbial isolates in their study were unable to produce siderophores.

Evaluation of local popular varieties for effective nodulation

Studies conducted at 45 DAS in Expt I towards the Nitrogen fixation responsiveness of four popular soybean varieties viz JS – 335 PK - 1029, MACS – M 124, LSB -1, with commercial biofertilizers resulted in higher nodule number (21.0), nodule weight (0.271g), Plant dry weight (15-163kg) and more/of total shoot (2.49%) compared to RDF treatment Viz T_2, T_5, T_8, T_{11} and control (Table 5). Further a positive influence of *Brady rhizobial* inoculation was evident in all the four varieties though JS – 335 was found to be superior. Results obtained from inoculation with this commercial biofertilizers indicated that certain varieties and rhizobial isolate combinations will respond better to symbiotic association. It is therefore important to consider the interaction of host and rhizobial isolate in selecting an appropriate soybean variety for commercial production, similar results were obtained by Senaratne *et al.* (1987) with reference to increased growth parameter in soybean by rhizobial strains.

In the Expt-2, nine *Brady rhizobium* isolates were tested for nodulation efficiency in an inoculum responsive popular soybean variety JS -335. The plants of JS – 335 inoculated with SBR -8 (T_5) had significantly higher plant dry weight (15.937 g) compared to control.

Inoculants with SBR – 5, SBR – 6, SBR – 7, SBR – 11 , and CRIDA soyo 809, on treatments T_2, T_3, T_4, T_8 and T_{11} also showed a positive response towards above other (Table 5). The influence of the Brady rhizobial isolates on growth parameters was in the order SBR 8 > SBR 6 > SBR 11 > CRIDA soyo 809. Further, the total Nitrogen content of plants varied from 2.11 to 2.94%, with a higher total N content of

Table 5. Effect of brady rhizobium inoculation on nodule number, nodule dry weight (g pl^{-1}), plant Dry weight (g pl^{-1}) plant dry weight (g pl^{-1}) Total Nitrogen content (%), at 45 DAS indifferent varieties of soybean

Treatments	Nodule No/Plant	Nodules dry weight(g pl1) Shoot+ root		Dry weight (g plant $^{-1}$) Nodule	Total	Total nitrogen content %
T1 (JS – 335 + CRIDA Soya 809	21.0	0.271	15.613	0.271	15.884	2.49
T2 (JS -335 + RDF)	12.66	0.121	9.040	0.121	9.161	2.38
T3 (JS -335 Control	5.33	0.034	5.933	0.034	5.965	2.23
T4 (PK 1029 + CRIDA Soya 809)	17.66	0.231	12.477	0.231	12.708	2.45
T5 (PK – 1029 + RDF)	10.0	0.087	8.910	0.087	8.997	2.34
T6 (PK 1029 Control	4.66	0.033	3.587	0.033	3.620	2.09
T7 (MACS 124 + CRIDA Soya 809)	14.66	0.117	12.72	0.117	12.884	2.42
T8 (MACS 124 + RDF)	7.33	0.068	6.710	0.068	6.778	2.26
T9 (MACS 124 Control)	5.66	0.048	4.673	0.048	4.721	2.19
T10(LSB 1 + CRIDA Soya 809)	19.0	0.202	11.367	0.202	11.569	2.46
T11 (LSB 1+ RDF)	10.0	0.105	9.023	0.105	9.128	2.36
T12 (LSB -1 Control)	5.0	0.032	3.587	0.32	3.619	2.17
*SEM ±	0.732	0.020	0.339	0.020	0.359	0.023
CD at 5%	2.138	0.060	0.992	0.060	1.052	0.068

S.E.M = Standard Error Mean CD = Critical Difference

Table 6. *Effect of different Brady rhizobial isolation on nodule number, dry weight (g pl^{-1}) dry weight (g pl^{-1}) total N content (%). Available Nitrogen in soil (kg ha^{-1}) at 45 DAS in soybean and microbial populations.*

Treatment	Nodule no/plant	Nodule dry. wt. g/plant	Shoot root	Nodule	Total	Total N content (%)	Available N in soil (kg/ha	Bacterial project (cfu x 10^6 g soil)	Rhizobial projects (cfu 10^2/ g soil	Actinomy-cetes (cfu x 10^3/ g soil	Fungal project (cfu x 10^3/ g soil
T_1 (JS-335 + SBR-4)	25.33	0.266	12.117	0.266	12.384	2.57	206.402	47.067	20.067	17.667	15.867
T_2 (JS-335 +SBR-5)	21.0	0.166	10.583	0.166	10.749	2.50	201.020	43.567	16.900	12.433	11.00
T_3 (JS-335 + SBR-6)	22.0	0.309	11.073	0.309	11.382	2.48	203.033	45.767	18.467	15.167	15.533
T_4 (JS-335, + SBR –7)	20.66	0.195	12.763	0.195	12.958	2.33	202.068	37.367	15.867	12.867	13.200
T_5 (JS-335 + SBR-8)	30.00	0.456	15.927	0.456	16. 383	2.94	213.998	50.60	22.467	16.733	20.400
T_6 (JS-335 + SBR-9)	17.33	0.185	10.667	0.185	10.852	2.45	198.692	39.5	13.067	11.50	13.433
T_7 (JS 335 +SBR-10)	14.66	0.110	9.723	0.110	9.833	2.41	197.385	35.433	14.867	13.067	14.233
T_8 (JS 335 + SBR-11)	22.0	0.229	11.777	0.229	12.006	2.51	205.147	43.967	17.533	13.567	12.167
T_9 (JS 335 + SBR -13)	14.33	0.144	11.927	0.144	12.071	2.41	198.821	37.533	13.167	13.700	9.733
T_{10}(JS335) Control	5.33	0.035	5.513	0.035	5.548	2.11	197.053	27.067	7.833	8.767	10.367
T_{11} (JS 335 + CRIDA Soy 809)	22.0	0.211	14.570	0.211	14.781	2.51	209.432	44.367	18.267	13.867	15.967
S. E m +/-	1.029	0.019	0.423	0.019	0.442	1.054	0.442	1.330	0.988	0.956	0.972
CD at 5%	3.02	0.057	1.241	0.057	1.298	0.161	1.299	3.902	2.898	2.803	2.852

S.E.M = Standard Error Mean CD = Critical Difference

2.94% in JS – 335 inoculated with SBR – 8 (T_5), (Table 6). The inoculation with the Brady rhizobial isolates also led to an increase in available soil N. The initial soil N content of 188 kg / ha) was raised to 213.98 ha^{-1} in JS 335 with SBR–8 inoculation (Table 6). The percentage increase in available soil N content over initial N content is 13.8% while it was 2.1% more, when compared with inoculation with CRIDA soya 809.

The fact that inoculated soybean plants had a higher N content than control plants indicates the presence of an active N fixing system that varies with the rhizobial strains present in the soil. Similar results in increased soil N control by rhizobial inoculations were obtained by Daterao *et al.* (1980) in green gram and Rashid *et al.* (1999) in groundnut.

The strong responses in nodulation and growth of soybean to *Brady rhizobium* biofertilizers application showed that the root nodule symbiosis plays a very important role in growth and performance of soybean. Though several methods are in use for assessment of effectiveness of Bradyrhizobial inoculants, an accurate measurement of N fixation is essential for selection of a correct suitable rhizobial isolate. In soybean, % N content or shoot dry weight, offer the best assessment parameters for symbiosis.

The SBR-8 inoculation in JS -335 increased the rhizospheric soil microflora at 45 DAS. It resulted in a higher total bacterial population of (50.60 x 10^6/g soil), Brady rhizobial population of (22.467 x 10^2/g soil) and fungal population of (20.40 x 10^3/g soil) except for actinomycetes population , which was more influenced by SBR-4 (T_{11}) inoculation (17.667 x 10^3/g soil), (Table 5). SBR–8 is more efficient in improving soil microbial population in addition to its improvement of plant growth parameters.

Conclusion

The present study thus indicates that, there is a significant improvement in nodulation, dry matter production, Soil microbial population, nutrient uptake with *Brady rhizobium* inoculation in soybean. It further showed that inoculation with Brady rhizobial isolates obtained from soybean plant nodules is also effective in improving the plant growth parameters. SBR – 8 isolate demonstrated increased dry matter production and

symbiotic N responsiveness. It may have the potential for effective biofertilizers development for the soybean plants. Thus the Brady rhizobial isolate obtained from Utnoor of Adilabad district one more effective in promoting better soybean growth than other isolates.

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