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A study on evaluation of nitrogen fixation potential in soybean cultivar using commercial and indigenous strains

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ABSTRACT

Earthen pots experiments were conducted for the evaluation of nitrogen fixing potential of three indigenous Bradyrhizobium japonicum isolates and one commercial inoculum (Narmada biofertilizer) in three commonly grown soybean cultivars viz., JS-335, JS-9305 and JS-7322. Nitrogen fixation was assessed in terms of root nodules number, root and shoot length and their dry weight. Results indicate that the indigenous isolates were more efficient than commercial isolate. When the indigenous isolates of B. japonicum were used, NOPP increased significantly in comparison to commercial inoculum. Similarly, indigenous isolates also showed significantly higher values in the case of root/shoot length its dry weight over commercial isolate. Although this is a preliminary study, it appears from the results that the indigenous strains of B. japonicum are more promising and efficient as compared to commercial inoculum.

Key Words: *Bradyrhizobium japonicum*, Yeast Extract Mannitol agar, Nodule occupancy per plant.

INTRODUCTION

One of the agricultural and environmental importance of legumes is their ability to establish symbiosis with nitrogen fixing bacteria, collectively known as rhizobia. Since nitrogen is a limiting nutrient for growth and yield of crops, rhizobia have a direct role to play in its supply to the growing plants. [1]. *Bradyrhizobium japonicum* (previously classified *as Rhizobium japonicum)* is defined as gram-negative α-proteobacterium capable of forming root nodules on soybean, mediated by *nod* genes [2, 3]. The ability to form nodules has been found to be highly host-specific for different species of rhizobia [4]. Direct consumption of soybean is very limited. Around 35% of the beans production is traded in the world market. Soybean is an important

global crop, providing oil and protein. Above 80% of the global soybean output is crushed worldwide to obtain oil and meal. Soybean in India mainly as a kharif crop and belongs to the family Leguminoceae and sub-family Fabiaceae. Soybean production is mainly confined to Madhya Pradesh (also known as soybean bowl of India), Maharashtra, Rajasthan, Andhra Pradesh, Karnataka, Uttar Pradesh and Chhattisgarh [5]. Though productivity of soybean is highest in Rajsthan state but Madhya Pradesh tops the list in crop acreage. The whole Malwa region including Indore, Ujjain, Ratlam, Dhar and Mandsour districts contributes to the soybean production of Madhya Pradesh. Soybean growing districts are grouped according to Yield Index. It has become the major crop of the area and according to Soybean Oil and Processors Association (SOPA), Indore report there is marked increase in production every year [6]. Understanding the diversity and beneficial activities of plant-bacterial association in agro ecosystems is important for sustainable crop production. Keeping in view the economic value of the crop, the suitability of region and concern for sustainable development the present study was carried out with an objective to compare indigenous and commercial bacterial inoculum for enhancing nitrogen fixation potential of three commonly grown soybean cultivars.

MATERIALS AND METHODS

Survey for plant collection:

Based on the preliminary surveys and soil conditions, agricultural fields with cultivars JS-335, JS-9305 and JS-7322 were selected. The sites for plant collections were situated on the peripheral region of Ujjain district and were away from each other at least by about 10 km. For nodule collection, soybean plants were excavated carefully avoiding any injury and were brought in the laboratory. Adhering soil was removed from root and root nodules were washed under a gentle stream of water.

Isolation of root nodule bacteria:

Bacteria were isolated from the root nodules. Healthy and pinkish root nodules were used for isolation. Procedure given was followed for isolation [7]. Firstly, root nodules were washed with tap water to remove soil debris and then soaked for 5-6 minutes in the 0.1% $HgCl_2$ with the help of sterile forceps. Nodules were then rinsed 3 to 4 times using sterilized distilled water and immersed in alcohol to break the surface tension and to remove air bubbles from the tissue. After 2-3 minutes, nodules were crushed using sterilized glass-rod in small aliquot of sterilized water and the suspension was streaked on the Yeast Extract Mannitol Congo Red (YEM-CR) agar medium in Petri-dishes in triplicates. Plates were incubated at 27°C for 5-7 days. Thereafter, the emerging bacterial colonies were picked up and transferred to YEM-CR slants. Commercial (Narmada Biofertilizer) inoculum was purchased from the MP Agro. Ltd., Ujjain.

Identification of bacterial isolates:

Identification of bacterial species was done following Bergey's Manual of Determinative Bacteriology [8] and growing them on YEM-CR medium.

Broth culture:

All four isolates were grown in the yeast extract mannitol broth for 7 days at 27±2ºC. *Composition of medium:* Mannitol-10 gram, K_2HPO_4-0.5 gram, $MgSO_4.7H_2O$- 0.3 gram, NaCl- 0.1 gram, Yeast Extract-0.5 gram, Distilled Water (D/W) - 1000 ml [9].

Experimental set-up:
Twenty five seeds of each cultivar were immersed in 0.1% $HgCl_2$ for surface sterilization and then soaked in distilled water and placed in the sterilizer Petri-plates with moist filter paper. After proper germination, 20 healthy germinated seeds of each cultivar were sown in each pot. Pots were 20x20x30 cm in size and in these 6 kg of sterilized soil along with inoculum of the *B. japonicum* was added in the ratio of 1:60. Fertilizer was also added in the pots as follow: KH_2PO_4-9.37g, KCl- 08.09g, $MgSO_4$ $7H_2O$-01.07g, $ZnSO_4.7H_2O$-01.00g, $(NH_4)_6$, MO_2-$4H_2O$-0.04g, d/w-1000ml. Water was added as and when needed. After 30-35 days of sowing ten plantlets were removed and washed with water and number of root nodules, shoot length, root length, shoot and their dry weight was taken and average value of ten plantlets were calculated. For each cultivar 4 pots were taken with triplicate and overall 36 pots were used.

***Statistical analysis*:**
The data were subjected to analysis of variance using the statistical program SPSS- V-0.1, and the significance was calculated at 5% and 1% while Duncan's Multiple Range Test (DMRT) was followed for the data as indicated in the table.

RESULTS

Individual colony of *Bradyrhizobium japonicum* isolates was observed for the size, color and shape after growth on YEM-CR plates for 7 days at 27±°1C. Morphological characters of all four isolates are shown in the **Table 1**. All the four isolates are showing the gram negative appearance with gram stain.

Nodule occupancy per plant (NOPP): The root nodule formation varied with different isolates and was different in different cultivars of soybean. B3 isolate produced maximum nodules (12.75) with JS-335 variety, the highest nodule formation in JS-9305 was with B8 (12.75), while it was maximum with B10 isolate in JS-7322 cultivar (13.00).

The commercial inoculum was comparatively poor in nodulation in all the cultivars of soybean and maximum nodule formation was in JS-9305 (9.75) which is very little in compare to others as mentioned in the **Table-2.**

Root length: In general, there was an increase in root length with bacterial inoculation as compare to non-inoculation (un-inoculated). However the increase was comparatively more in case of indigenous isolates than commercial inoculum. It was observed that isolate B3 was effective in case of cultivar JS-335 (7.5 cm) while B8 and B10 were effective for cultivar JS-9305 (7.5cm) and JS-7322 (7.8cm) respectively **(Table-2).**

Shoot length: A similar trend as seen in root length was observed in shoot length also. Again the commercial inoculum did not show any significant increase as compare to indigenous isolate in all the three test varieties of soybean. Also B3 was found effective in JS-335, B8 in JS-9305 and B10 in JS-7322 **(Table-2).**

Table 1. Morphological characteristics of isolates

S. No.	Colony Characters	B3	B8	B10	Narmada Biofertilizer
1	Colony color	White	White	White	White
2	Absorption of CR	Negative	Negative	Negative	Negative
3	Transparency	Opaque	Opaque	Opaque	Opaque
4	Nature of Colony	Glistening	Glistening	Glistening	Glistening
5	Margin of colony	Entire	Entire	Entire	Entire
6	Surface of Colony	Smooth	Smooth	Smooth	Smooth
7	Gram Staining	Gram negative	Gram negative	Gram negative	Gram negative

Table 2. Comparative study of different rhizobial isolates on nitrogen fixation potential

Soybean cultivars	Isolates	NOPP	Nodules Dry Weight	Root length (cm)	Root Dry weight	Shoot Length (cm)	Shoot Dry Weight
JS-335	B3	12.66±1.3^{a}	0.75±0.12 ^{a}	7.75±1.6^{a}	0.47±0.05^{a}	9.1±2.1^{a}	0.52±0.06^{ab}
	B8	11.5±1^{a}	0.7±0.14^{ab}	7.25±2.1^{a}	0.45±0.07^{a}	9±3^{a}	0.49±0.05^{ab}
	B10	11.75±1.5^{a}	0.73±0.1^{ab}	7±1.1^{a}	0.4±0.03^{a}	8.8±1.8^{a}	0.46±0.04^{a}
	Commercial inoculum	9.5±0.8^{b}	0.65±0.15^{c}	6.5±2.5^{b}	0.37±0.09^{b}	8±1.6^{b}	0.41±0.05^{b}
JS-9305	B3	12±1.8^{a}	0.73±0.18^{a}	7.25±2.9^{a}	0.45±0.05^{a}	9±0.7^{a}	0.51±0.07^{ab}
	B8	12.75±2^{a}	0.77±0.11^{ab}	7.75±2.1^{a}	0.49±0.06^{a}	9.75±2.6^{a}	0.54±0.05^{ab}
	B10	12.25±1.3^{a}	0.73±0.13^{ab}	7.25±1^{a}	0.46±0.03^{a}	9.5±2.2^{a}	0.52±0.06^{a}
	Commercial inoculum	9.75±1.7^{b}	0.65±0.17^{c}	6.5±2^{b}	0.35±0.08^{b}	8.25±1.3^{b}	0.43±0.04^{b}
JS-7322	B3	12.25±1.6^{a}	0.7±0.2^{a}	7±1.3^{a}	0.45±0.06^{a}	9.5±2.6^{a}	0.53±0.05^{ab}
	B8	12.75±1.8^{a}	0.75±0.18^{ab}	7±1.5^{a}	0.46±0.04^{a}	9.25±1.3^{a}	0.53±0.08^{ab}
	B10	13±1.1^{a}	0.8±0.13^{ab}	7.8±2.6^{a}	0.5±0.05^{a}	9.47±2.1^{a}	0.6±0.06^{a}
	Commercial inoculum	9.75±0.9^{b}	0.66±0.16^{c}	6±1.5^{b}	0.36±0.09^{b}	8.75±1.9^{b}	0.5±0.03^{b}

NOPP (isolates $F_{4,8}$ = 19.39, P<0.001), Dry weight of nodules (isolates $F_{4,8}$ = 6.931, P<0.01), Root length (isolates $F_{4,8}$ = 4.857, P<0.05), Root dry weight (isolates $F_{4,8}$ = 8.172, P<0.001), Shoot length (isolates $F_{4,8}$ = 4.55, P<0.05). The mean ± SE values for three replicates not followed by common lower-case letters are significantly different at P< 0.05; accept shoot dry weight, as determined by Duncan's Multiple Range Test.

DISCUSSION

The response of inoculum with respect to nodule formation (NOPP)/ root and shoot length and their dry weight was reflected in dry matter production as well. As revealed from the **Table-2** that isolates of *B.japonicum* are effective in terms of nitrogen fixation parameters that directly related to better growth of the crop plants. It also reveals that the inoculum specificity with the crop cultivars. As was evident in the present study the indigenous strain B3 resulted in a better growth in cultivar JS-335, while B8 was more effective in JS-9305 and B10 was best for JS-7322. All the parameters such as NOPP, root/shoot length and their dry weight are reflected with same trend. However, the indigenous strain has a performance edge over the commercial strain. The shoot dry weight of plant harvested in floral initiation is the generally accepted criteria for nitrogen fixing effectiveness, but nodule number and dry weight may also be employed more refined evaluation [9]. The introduction and persistence of strain(s) is affected by a number of abiotic factors including high soil temperature and pH. Populations of *Bradyrhizobium* species vary in their tolerance to these stresses [10, 11 and 12]. Associations of symbiotic partners are

quite specific, so when legume species are introduced into new regions it becomes necessary to inoculate them with the appropriate rhizobia in order to establish an effective symbiosis [13]. An attempt to introduce superior important strain into soil already inhabited by effective indigenous rhizobia may sometimes lead to a great failure and it is reflected in our study [9, 14]. It is well known that he microorganisms and the nutrients present in the raw materials are very helpful in improving soil health. There are different types of biofertilizers available that their differences are mainly the raw materials used, forms of utilization and the sources of microorganisms [15]. It is revealed from this study that, to attend effective nodulation and best productive results at least three major concerns should be kept in mind by the researchers and farmers. First the edaphic conditions of the area concern, second the cultivar specificity and the third is the presence of indigenous strains for inoculum if any.

Our research should concentrate more on improvement of India strains and their usage on field scale rather than the introducing an entirely new strain without prior testing its efficiency in the new edaphic and climatic conditions.

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