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RESEARCH ARTICLE

STUDIES ON THE EFFICIENCY OF N_2 FIXATION BY *Bradyrhizobium japonicum* ISOLATED FROM ROOT NODULES OF SOYBEAN GROWN UNDER SEMIARID TROPICS OF TAMIL NADU

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ABSTRACT

The competitive nodulation ability of a *Bradyrhizobium* strain is an important property, as strains must compete with other Bradyrhizobia in the rhizosphere for nodulation sites on the host plant. If inoculants strains are to succeed, they must have the ability of competitiveness as well as being effective in symbiosis. The establishment of effective nodulation of soybean plant can be enhanced by using effective and competitive strains of *Bradyrhizobium*. In the present study 30 isolates of *Bradyrhizobium japonicum* obtained from semiarid locations were compared for their N_2 fixing efficiency based on IAA production, EPS production, nodulation, nodule ARA activity and nodule N content. The isolate obtained from Tiruchengodu SBJ-14 produced maximum of IAA, EPS and ARA activity and this isolate also recorded the highest number of 30.00 nodules plant^{-1} ARA activity of 215.00 n moles C_2H_4 formed h^{-1} g^{-1} and nodule nitrogen content of 7.44%.

INTRODUCTION

Nitrogen is one of the major important nutrients essential for plant growth. The economic and environment importance of legume crops is largely due to their ability to fix atmospheric dinitrogen in a symbiosis with specific bacteria *(Rhizobium or Bradyrhizobium species)*. *Bradyrhizobium japonicum* is a slow growing root nodule symbiont, which is widely used as an inoculant in soyabean fields through out the world. Generally, soybean inoculated with *Bradyrhizobium japonicum* forms highly effective nodules and frequently increased soybean yields, especially in fields where soybeans are cultivated for the first time. (Caldwell and Vest, 1970)

The major problem of soybean inoculation is existing indigenous strains in the field may often suppress the introduced inoculants strains applied to soybeans subsequently. Therefore, it is necessary that the highly effective introduced strain has also the capacity to compete with the resident ineffective rhizobia in the soil. (Dowling and Broughton, 1986)

Environmental factors such as temperature, moisture, acidity and salinity and several chemical component of the soil are the limiting factors of the Rhizobium-legumae symbiosis. Both establishment and activity of the legume-rhizobium symbiosis are known to be sensitive to drought stress (Kirda *et al.,* 1989).

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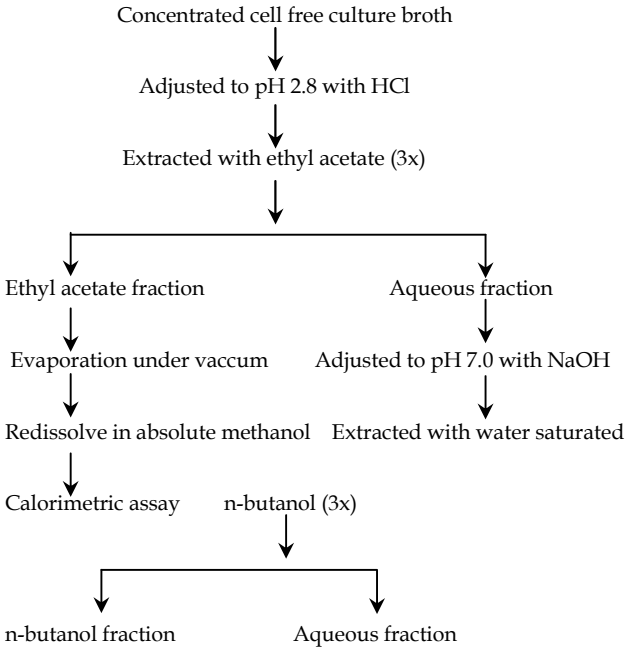
Effectiveness of the symbiosis is measured either directly by determining the amount of nitrogen fixed or indirectly by measuring the plant dry weight. Hardy *et al.* (1968) gave the methodology, characteristics and application of the sensitive ARA for measurement of N2 fixation rate by nitrogenase preparations and bacterial cultures in the laboratory and by legumes and free living bacteria in situ. The nitrogen fixing efficiency of the *Rhizobium* isolates is an attribute for selecting strains for crop improvement programme (Grant and Purdon, 1997; Narendra Kumar *et al.* 1996; Kirichenko and Malichenko, 2000). The objective of the present study is to analyze nitrogen fixing efficiency of *Bradyrhizobium* isolates obtained from various soybean grown semi arid tropics of Tamil Nadu.

MATERIALS AND METHOD

The soybean root nodule isolates obtained from 30 different locations of semiarid tropics were identified based on their size, shape, gram reaction, colony morphology, growth on YEMA with bromothymol blue (Norris, 1965), growth on Hofer's alkaline medium and they were named as *Bradyrhizobium japonicum* SBJ-1 to SBJ-30. Then the isolates were screened for nitrogen fixing efficiency based on IAA production, EPS production, nodulation, nodule ARA activity and nodule N content.

Quantitative estimation of Indole Acetic Acid (IAA): The yeast extract Mannitol broth in 100 ml quantities were prepared and supplemented with DL-Tryptophan, at a concentration of 100 mg/litre after sterilization. This

was followed by the addition of standard inoculum ($1x10^{7}$ cells/ml) of the isolates and incubated at 30°C under dark for a period of 7-12 days inorder to prevent the photo-inactivation of the biologically active compounds. The solution was centrifuged at 7000rpm for 30 minutes and the supernatant was reduced to 50ml volume by flask evaporation under vacuum and IAA extracted in to ethylacetate and n-butanol by the procedure followed by Tien *et al.,* (1979) as stated below.



Estimation of IAA: IAA in the methanol fraction was determined by employing salper reagent. To 1.5 ml distilled water in a test tube 0.5 ml of methanol residue was mixed, 4 ml fresh Salper reagent was rapidly added and kept in darkness for one hour and read in calorimeter at 535 nm. From a standard graph prepared with known concentrations of IAA, the quantity of IAA in the filtrate was calculated.

Quantitative estimation of Exopolysacchrides produced by the isolates: 2 ml of the inoculum was added to 100 ml of YEM liquid medium and incubated on Psychrotherm incubator Shaker at 28°C for 72 hours. The cells are harvested by centrifugation.

Water soluble polysacchrides (WSP): 60 ml of isopropyl alcohol was added to 20 ml of the supernatant fraction and let stand at 4°C overnight to precipitate WSP. It was collected by filtering through Whatman No.42 filter paper and dried in an incubator at 60°C till a constant weight obtained. (Sutherland and Wilkinson, 1971).

Nodule production efficiency of the isolates: The efficiency of the isolates to produce nodules were studied by Leonard jar experiment described by Somasegaran and Hoben (1985).

Sowing seeds: Fair surface sterilized seeds of soybean were sown in sand aseptically and watered with sterile distilled water, after seed germination only two seedlings were retained per jar after removing the surplus with sterile forceps. The sand surface was covered with 1-2 cm deep layer of 3-6 mm size sterile limestone gravel.

Inoculation : All the isolates which showed positive results for nodulation were considered. Young culture of *Bradyrhizobium* isolates grown on YEMA slope was suspended in a sterile water to make a turbid solution. 1 ml of the suspension was poured around the seedling soon after the germination of the seed. Three replications were maintained for each treatment.

Harvesting: After 45 days, the plants were carefully removed along with their roots and the total number of nodules plant-1 was counted of recorded.

Estimation of nitrogenase activity by acetylene reduction assay method: One gram of root nodules were placed in 65 ml serum vials and closed with rubber stoppers. With the sterile disposable syringe, 6.3 ml of air from the serum vial was evacuated and 6.3 ml of acetylene gas was injected and these bottles were incubated at 28°C for one hour. At the time of assay, using a sterile disposable syringe, 0.5 ml of the gas sample was with drawn after flushing twice and injected into gas chromatograph and tested for ethylene production. The factor 0.006 was arrived by injecting pure ethylene gas. The nitrogenase activity was expressed as a mole of ethylene produced per gram of nodules per hour (Hardy *et al.,* 1968).

$$\text{Nitrogenase activity} = \frac{\text{Peak height in mm x attenuation x range x 0.006 x volume of acetylene gas injected}}{\text{Hours of incubation x volume of ethylene gas injected into gas chromatograph}}$$

Estimation of N content of root nodules: The nitrogen content of the root nodule was estimated by following Microkjeldahl method diacid extraction H_2SO_4: $HClO_4$ in the ratio of 5:2 (Humphries, 1956).

RESULT AND DISCUSSION

In our study, all the thirty isolates of *Bradyrhizobium japonicum* (SBJ-1 to SBJ-30) produced IAA in tryptophan supplemented YEM broth and the quantity ranged from 0.65 to 6.10 µg ml^{-1} of the culture medium. Among the 30 isolates tested, the isolate SBJ-14 produced maximum IAA of 6.10 µg ml^{-1}, followed by SBJ-23, SBJ -2 and SBJ-10. Bacteria belonging to the genera *Rhizobium* and *Bradyrhizobium* secrete copious amounts of exopolysacchides when they are cultured invitro, several reports indicated that, the exopolysacchides may play an important role in the process by which these bacteria nodulate legumes and a close relationship between exopolysacchride production and infectivity (Muller *et al.,* 1988). All the thirty isolates produced EPS in the range from 12.25 to 320.40 µg ml^{-1}. The isolate SBJ-14 produced the maximum EPS of 320.40 µg ml^{-1} followed by SBJ-23, SBJ-2 and SBJ-10.

Inoculation with *Bradyrhizobium japonicum* increased nodule mass significantly in soybean (Morote *et al.,* 1990). Daramola *et al.,* (1994) reported that soil inoculation with *Bradyrhizobium japonicum* resulted in more nodules, more uniform distribution

Table 1. Screening of Efficiency of the isolates from root nodules of soybean grown under semiarid tropics of Tamil Nadu

S. No.	Locations	Isolate	IAA	EPS	No. of nodules plant $^{-1}$	ARA	N content %
1	Attayyampatti	SBJ-1	1.30	120.30	12.00	110.40	2.65
2	Sankagiri	SBJ-2	4.95	280.00	20.00	187.00	4.95
3	Idappadi	SBJ-3	0.86	41.30	11.00	114.75	3.65
4	Tharamangalam	SBJ-4	1.95	127.20	13.00	117.20	4.35
5	Mallur	SBJ-5	3.10	104.10	17.00	142.00	3.25
6	Malliyakaral	SBJ-6	2.00	18.70	11.00	100.25	3.00
7	Attur	SBJ-7	3.15	110.70	18.00	145.00	3.25
8	Vazhappadi	SBJ-8	3.00	200.00	18.00	140.00	3.20
9	Konganapuram	SBJ-9	0.65	63.75	9.00	93.00	2.95
10	Poolampatti	SBJ-10	3.85	265.00	20.00	176.25	3.50
11	Rasipuram	SBJ-11	0.76	67.00	10.00	98.75	2.98
12	Namagiripettai	SBJ-12	3.79	137.75	17.00	130.00	3.00
13	Kalangani	SBJ-13	0.85	37.00	8.00	94.25	2.25
14	Tiruchengode	SBJ-14	6.10	320.40	30.00	215.50	7.44
15	Erumaippati	SBJ-15	2.47	50.00	14.00	120.00	3.15
16	Mallasamudram	SBJ-16	3.45	250.00	18.00	180.00	4.80
17	Elachipalayam	SBJ-17	1.75	26.00	10.00	98.00	3.25
18	Jedarpalayam	SBJ-18	1.20	18.60	12.00	96.25	2.95
19	Mohanur	SBJ-19	2.57	48.00	14.00	125.00	3.10
20	Velakavundanpatti	SBJ-20	1.00	54.00	9.00	95.25	2.50
21	Mallapuram	SBJ-21	2.00	18.00	11.00	110.25	2.95
22	Nallampalle	SBJ-22	2.15	28.70	11.00	99.25	2.50
23	Papireddipatti	SBJ-23	5.25	310.40	21.00	197.00	5.75
24	Adiyaman Kottai	SBJ-24	2.61	55.00	12.00	140.45	3.50
25	Morappur	SBJ-25	1.90	14.20	12.00	96.25	3.00
26	Singarapettai	SBJ-26	2.45	43.20	13.00	110.00	3.00
27	karimangalam	SBJ-27	1.75	32.25	10.00	96.25	2.25
28	Samalpatti	SBJ-28	1.95	35.00	10.00	94.25	3.00
29	Ballurpettai	SBJ-29	0.85	37.00	8.00	94.25	2.00
30	Pennagaram	SBJ-30	1.80	12.25	13.00	90.25	3.25

on the root and greater N_2 fixation. In out present study the isolate SBJ-14, isolated from Tiruchengodu of Namakkal District recorded the highest number of 30 nodules plant^{-1}, ARA activity of 215.50 n moles C_2 H_2 formed h^{-1} g^{-1} and nitrogen content of the nodule was 7.44 per cent.

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