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STUDIES ON EFFICIENCY OF N2 FIXATION BY B*radyrhizobium japonicum* ISOLATED FROM ROOT NODULES OF BLACK GRAM GROWN UNDER PROBLEM SOILS (SALINE)

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ABSTRACT

Growth, nodulation and yield response of Black gram [*Vigna mungo* (L.) Hepper] to inoculation by *Bradyrhizobium japonicum*. The competitive nodulation ability of a *Bradyrhizobium* strain is an important property, as strain must compete with other Bradyrhizobia in the rhizosphere for nodulation sites on the host plant. If inoculant strain are to succeed they must have the ability of competitiveness as well as being effective in symbiosis. The establishment of effective nodulation of green gram plant can be enhanced by using effective and competitive strains of *Bradyrhizobium*. In the present study 30 isolates of *Bradyrhizobium japanicum*. obtained from saline 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 vallampadugai (GPJ-18) produced maximum of IAA, EPS and ARA activity and this recorded the highest number of 25.00 nodules plant-1 ARA activity of 202.50 moles C_2H_4 formed $h^{-1}g^{-1}$ and nodule nitrogen content of 5.75%.

INTRODUCTION

Nitrogen is one of the major important nutrient essential for plant growth. The economic and environment importance of legume crop 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 green gram fields throughout the world. Strain competitiveness is influenced by the genetic diversity of both symbiotic partners (Triplett & Sadowsky,

1992) and the soil environment in which nodulation occurs (Streeter, 1994). Brockwell *et al*., (1995) considered that inoculation is invariably futile in soils with populations greater than 1,000 rhizobia g-1 whereas in soils with smaller, less effective populations a response would depend on the ability of the inoculant to compete with rhizobia in the soil. The information enabling rhizobia to fix nitrogen by way of conventional nitrogenase is encoded in *nif* genes which are regulated by a complex set of processes (Dixon, 1987).The major problem of green gram inoculation is existing indigenous strains in the field may often suppress add 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 Broungton 1986). The environmental factors such as temperature, moisture, acidity, salinity and several chemical competence of the soil are the limiting factors of the Rhizobium legume symbiosis. 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 sensitive ARA for measurement of N_2 fixation rate of 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 Purdeon., 1997; Narandrakumar *et al.*, 1996; Kirichanko and Malichanko, 2000). However, there is evidence that a significant response to inoculation is possible in soils containing large numbers of established rhizobia when strains with both superior N_2-fixation efficiency and nodulation competitiveness are inoculated (Bradley *et al*., 1991; Hungria *et al.*, 1998).

MATERIALS AND METHODS

The Green gram root nodule isolates obtained from 30 different locations of saline areas were identified based on their size, shape, Gramreaction, Colony Morphology growth on YEMA with bromothymolblue (Norris, 1965), growth on Hofer's alkaline medium and they were named as *Bradyrhizobium japonicum* GBJ-I to GBJ-30. Then the isolates were screened for nitrogen fixing efficiency based on IAA production, EPS production, nodulation,

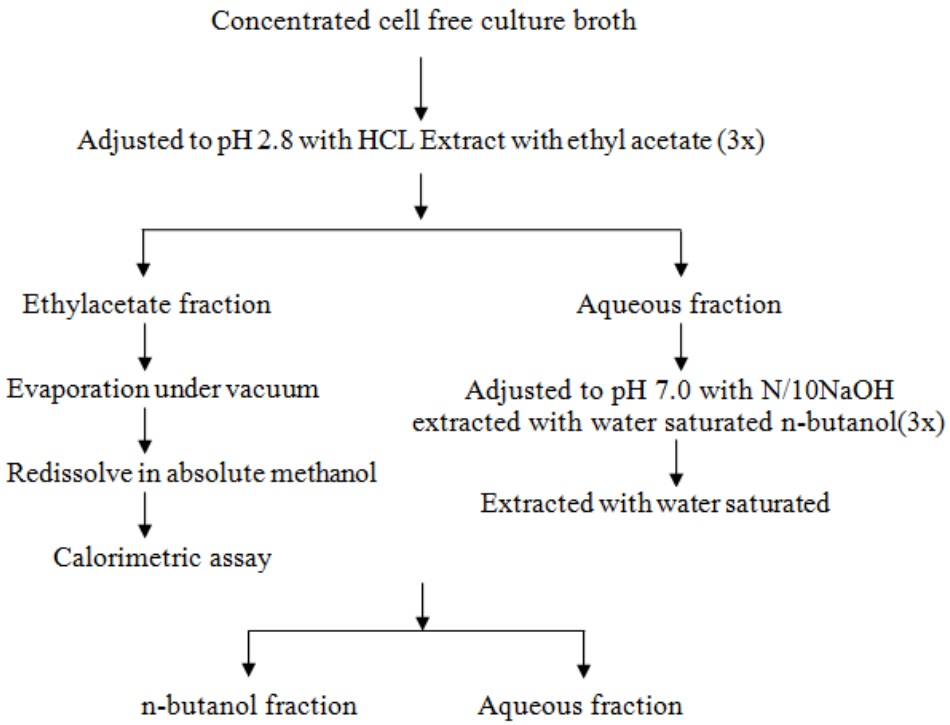
nodule ARA activity and nodule N content of N2 Fixation.

Quantitative estimation of Indole Acetic Acid (IAA)

The yeast extract mannitol broth in 100ml quantities were prepared and supplemented with DL-Tryptophan, at a concentration of 100mg/litre after sterilization. This was followed by the addition of standard inoculation 1X107 cells/m) 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 sulper regent. To 1.5 ml distilled water in a test tube 0.5ml of methanol residue was mixed, 4 ml fresh salper reagent was rapidly added and keptin darkness for one hour and read in calorimeter of 535nm. From a standard graph prepared with known concentration the quantity of IAA, in the filtrate was calculated. 1division=0.377µg of IAA.



Quantitative estimation of Exopolysaccharides produced by the isolates

To 100ml of the inoculam was added to 100ml of YEMA liquid medium and incubated on Psychrotherm them incubator shaker at 28℃ for 72 hours. The cells are harvested by centrifuging at 4000 for 10min.

Water soluble Polysaccharides (WSP)

60 ml of iso propyl alcohol was added to 20ml of the supernatant fraction and let stand at 4℃ overnight to precipitate WSP. It was collected by filtering through whatman No. 42 filter papter and dried in an incubator at 60℃ till constant weight obtained (Sutherland and Wilkingon, 1971).

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 add these bottles were incubated at 28℃ for one hour. At the time of assay, using a sterile disposable syringe, 0.5 ml of the gas sample was withdrawn 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).

Table 1: Screening of Efficiency of the isolates from root nodules of green gram grown under saline areas of Tamil Nadu

S. No	Locations	Isolate	IAA	EPS	No. of Nodules plant $^{-1}$	ARA	N Content
1.	Konayampattinam	GBJ-1	1.80	12.25	13.00	90.25	3.25
2.	Poompuhar	GBJ-2	0.85	37.00	8.00	94.25	2.25
3.	Mangaimadam	GBJ-3	1.95	35.00	10.00	96.25	3.00
4.	Thiruvengadu	GBJ-4	1.75	32.25	10.00	110.00	2.25
5.	Melaiyur	GBJ-5	2.45	43.20	13.00	96.25	3.60
6	Poraiyar	GBJ-6	1.90	14.20	12.00	110.00	2.25

7.	Tharangampadi	GBJ-7	2.61	55.00	12.00	96.25	3.00
8	Neithavasal	GBJ-8	5.25	310.40	21.00	140.45	. 3.00
9	Peruthottam	GBJ-9	0.15	28.70	11.00	170.25	3.50
10	Annapanpattai	GBJ-10	2.00	18.00	11.00	99.25	2.75
11	Kuravallur	GBJ-11	1.00	54.00	9.00	110.25	2.50
12	Semmangudi	GBJ-12	2.57	48.00	14.00	95.25	2.95
13	Kadavasal	GBJ-13	1.20	18.60	12.00	125.00	2.50
14	Edamanal	GBJ-14	1.75	26.00	10.00	96.25	3.10
15	Pichavaram	GBJ-15	0.95	110.50	12.00	100.50	2.00
16	Killai	GBJ-16	2.47	50.00	14.00	180.00	3.25
17	C. Mutlur	GBJ-17	17 5.30	220.00	30.00	120.00	4.80
18	Vallampadugai	GBJ-18	6.10	360.50	25.00	202.50	5.75
19	Sivapuri	GBJ-19	3.79	137.75	17.00	94.25	3.44
20	Portonova	GBJ-20	0.71	67.00	10.00	130.00	2.25
21	Sethiathope	GBJ-21	1.30	120.30	12.00	187.00	4.95
22	Vadalur	GBJ-22	0.65	90.75	9.00	176.25	4.75
23	Annamalainagar	GBJ-23	3.00	200.00	18.00	93.00	3.50
24	Pinnathur	GBJ-24	3.15	110.70	18.00	140.00	2.95
25	Devanampattinam	GBJ-25	2.00	18.70	11.00	145.00	3.20
26	T.S. Pettai	GBJ-26	3.10	104.10	17.00	100.25	3.00
27	Jayakondpattinam	GBJ-27	1.95	127.20	13.00	142.00	3.25
28	Samiarpettai	GBJ-28	0.86	41.30	11.00	117.20	4.35
29	Sembathinerupu	GBJ-29	4.95	250.00	20.60	114.75	3.65
30	Allivilagam	GBJ-30	1.30	120.30	12.00	167.00	2.95

$$\text{Nitrogenase activity} = \frac{\text{Peak height in mm} \times \text{attenation} \times \text{range } 0.006 \times \text{volume of gas injected}}{\text{Hours of incubation} \times \text{Volume of ethylene gas injected into gas chromatography}}$$

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).

In our study all the thirty isolates of *Bradyrhizobium japonicm* GBJ-1 to GBJ J-30 produced IAA in tryptophan supplemented YEM broth and quantity ranged from 0.15 to 6.10 mg m^{-1} of the culture medium. Among the 30 isolates tested, the isolate GBJ-18 produced maximum IAA of 6.10g ml^{-1}, followed by GBJ-23, GBJ-2 and GBJ-10. Bacteria belonging to the genera Rhizobium and *Bradhyrhizobium* secrete copious amounts of *exopolysacchardies* may plan an important role in the process by which these bacteria modulate legumes and a close relationship between exopolysaccharide production and infectivity (Muler *et al*., 1988). All the thirty isolates produce EPS in the range from 12.25 to 320.40 g ml^{-1} followed by GBJ-23, GBJ-2 and GBj-10. Incubation with *Bradhyrhizobium japonicum* increased nodule mass significantly in greengram (Movole *et al.*, 19990). Daramola *et al.*, (1994) reported that soil incubation with *Bradyrhizobium japonicum* resulted in more nodules, more uniform distribution on the root and greater N_2 fixation. In present study the isolate GBJ-14, isolated from vallampadugai of Cuddalore District recorded the highest number of 25 nodules plant-1, ARA activity of 202.50 n moles C_2H_2 formed h^{-1} g^{-1} and nitrogen content of the nodule was 5.75percent.

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