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Assessment of nitrogen fixing efficiency of Bradyrhizobial isolates from the coastal tracts of Cuddalore District

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Abstract
Salinization is one of the most crucial factors threatening agricultural land throughout the world. Approximately one third of the 260 million ha of irrigated land worldwide is affected by salinization (El-Akhal *et al*., 2013) [3]. Salinity limiting the productivity of crop plants because most of the crop plants are sensitive to salinity caused by high concentrations of salts in the soil, and the area of land affected by it is increasing day by day. Now we focused the native rhizobia from Cuddalore District. In this study, total thirty rhizobial isolates were tested for their ARA activity, N content and leghaemoglobin content of modules. Among the thirty isolates such as GNBJ- 4, GNBJ- 8, GNBJ- 9 and GNBJ- 28 produced ARA activity in the range of 188.25 to 215.40 n moles C_2H_4 formed $h^{-1}g^{-1}$, Nitrogen content in the range of 6.50 to 7.20% and leghaemoglobin content ranged from 0.185 to 2.110 nodule mg g^{-1}

Keywords: ARA activity, coastal area, leghaemoglobin & Soil nutrient

Introduction
The environment has long been known to influence symbiotic nitrogen fixation. The delicate balance between the host plant and the symbiont is disturbed even by mildly adverse conditions that have no effect on plant growth supported by soil nitrogen. Salt stress is one of the major types of environmental stress adversely affecting legume production in arid and semi-arid regions, particularly because these plants depend on symbiotic N_2 fixation for their nitrogen requirements (El-Sheikh and Wood, 1995) [4].

Salinity seriously constrains crop yield in irrigated agriculture throughout the world. Also, salinity is a serious threat to agriculture in arid and semi arid regions. Nearly 10 % of the world's land surface can be classified as endangered by salinity. Most of these areas are in the tropics and Mediterranean regions. Increasing salt concentration may have detrimental effects on soil microbes because of direct toxicity as well as through osmotic stress. Chloride and sulfate salts are predominant in saline soils. Plant growth, nutrient uptake and metabolism, and protein synthesis are all thought to be adversely affected by salt stress conditions. Retarded plant growth, resulting in reduced crop yield, has been reported by many investigators as a major cause of abnormal nutrient metabolism and impaired protein synthesis in plants under stress conditions.

The leguminous plants are required mostly neutral or slightly acidic soil for the growth, especially when they depend on symbiotic N_2 fixation and as well as more sensitive to salinity than their rhizobial counterparts and consequently, the symbiosis being more sensitive to salt stress than free living rhizobia. The strategies employed in the last few years to reduce the effect of salt stress on legume production have been focused on a selection of host genotypes that are tolerant to high salt conditions. Annually, biological nitrogen fixation is estimated to be around 175 million tonnes of which 79 per cent is accounted by terrestrial fixation. Vegame-*Rhizobium* symbiotic association observed to fix 60-100 kg N ha^{-1} $crop^{-1}$ (Anonymous, 2000) [1]. In the presence study was the selection of the efficient strain on the basis of nodule ARA, nodule Nitrogen and leghaemoglobin content.

Materials and Methods
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) [11]. Four surface sterilized seeds of groundnut were sown in sand in the leonard jar aseptically and watered with sterile distilled water, after seeds 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 sterile

limestone gravel.

A young culture of *Bradyrhizobium* isolates grown on YEMA slants 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. After 45 days, the plants were carefully removed along with their roots and the total number of nodules plant^{-1} were counted and recorded.

Estimation of Nitrogenase activity by Acetylene Reduction assay (ARA) activity

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, 0.5ml of 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) [6].

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

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) [8].

Estimation of Leghaemoglobin Content of Nodules

Preparation of tris-acetic acid buffer

0.1 N acetic acid (6.0 g l^{-1}) is adjusted to pH 4.0 with 0.2 tris M (hydroxy methane) methylamine (24.38 g l^{-1}).

Preparation of benzidine reagent

100 mg of benzidine was added to 0.5 ml of hydrogen peroxide and the volume was made upto 50 ml.

Determination (Sciffman and Lobel, 1970) [10]

The nodules were washed and weighed. They were crushed in tris- acetic acid buffer. The extract was centrifuged at 3000 x g for 20 min and supernatant 0.1 to 1.0 ml was taken, so as to get an absorbance reading between 0.2 to 0.4 and made up, each to final volume of 4.0ml by tris acetic acid buffer. Later, 2 ml of freshly prepared benzidine regent was added. The rate of colour formation was noted by observing the change in optical density using spectronic-20 spectrophotometer at 540 nm. A standard graph was prepared by plotting the absorbance at the end of 30 seconds against different concentrations (0.8 to 1.5 u,g ml^{-1}) of the ox-blood haemoglobin. The leghaemoglobin content of the test samples, was calculated from the standard graph and expressed in mg per g of nodules in fresh weight basis.

Results and Discussion

The 30 isolates were also screened for ARA activity and N content. The ARA activates showed by the isolates were ranged from 84.25 to 215.40 n moles C_2H_4 formed h^{-1}g^{-1}. The four isolates *viz.*, GNBJ-4, GNBJ-8, GNBJ-9 and GNBJ-28 produced ARA activity in the range of 188.25 to 215.40 n moles C_2H_4 formed h^{-1}g^{-1}.The isolate GNBJ-9 produced maximum of 215.40 n moles C_2H_4 formed h^{-1} g^{-1} of ARA (Table-1; Figure-1). The total Nitrogen content of groundnut nodules from 30 isolates are presented in the Table-2. Total nitrogen content ranged from 2.05 to 7.20%. Among the isolates, the four isolates GNBJ-4, GNBJ-8, GNBJ-9 and GNBJ-28 had nitrogen content in the range of 6.50 to 7.20%. The isolate GNBJ-9 had maximum nitrogen content of 7.20%. The increase in leghaemoglobin content of nodules obtained from the groundnut plants raised from inoculated strains was significant. Leghaemoglobin content ranged from 0.185 to 2.110 nodule mg g^{-1}. The leghaemoglobin content was found to be relatively high in nodules inoculated with strain GNBJ-9, this was followed by GNBJ-4, GNBJ-8 and GNBJ-28 (Table-3).

Table 1: Effect of inoculation of strains of Bradyrhizobia on Nodule ARA Activity of groundnut plants

S.No	Isolates	ARA (n moles C_2H_4 h^{-1} g^{-1}nodule)
1	GNBJ-1	100.75
2	GNBJ -2	98.00
3	GNBJ -3	105.76
4	GNBJ -4	188.25
5	GNBJ-5	118.00
6	GNBJ-6	99.30
7	GNBJ-7	156.75
8	GNBJ-8	198.10
9	GNBJ-9	215.40
10	GNBJ-10	148.20
11	GNBJ-11	90.10
12	GNBJ-12	127.00
13	GNBJ-13	136.75
14	GNBJ-14	120.50
15	GNBJ-15	149.00
16	GNBJ-16	175.00
17	GNBJ-17	110.00
18	GNBJ-18	138.40
19	GNBJ-19	140.00
20	GNBJ-20	100.10
21	GNBJ-21	156.20
22	GNBJ-22	178.20
23	GNBJ-23	180.00
24	GNBJ-24	124.50
25	GNBJ-25	96.20
26	GNBJ-26	147.50
27	GNBJ-27	84.25
28	GNBJ-28	190.20
29	GNBJ-29	164.50
30	GNBJ-30	174.20
	SED	1.54
	CD (P=0.05)	2.93

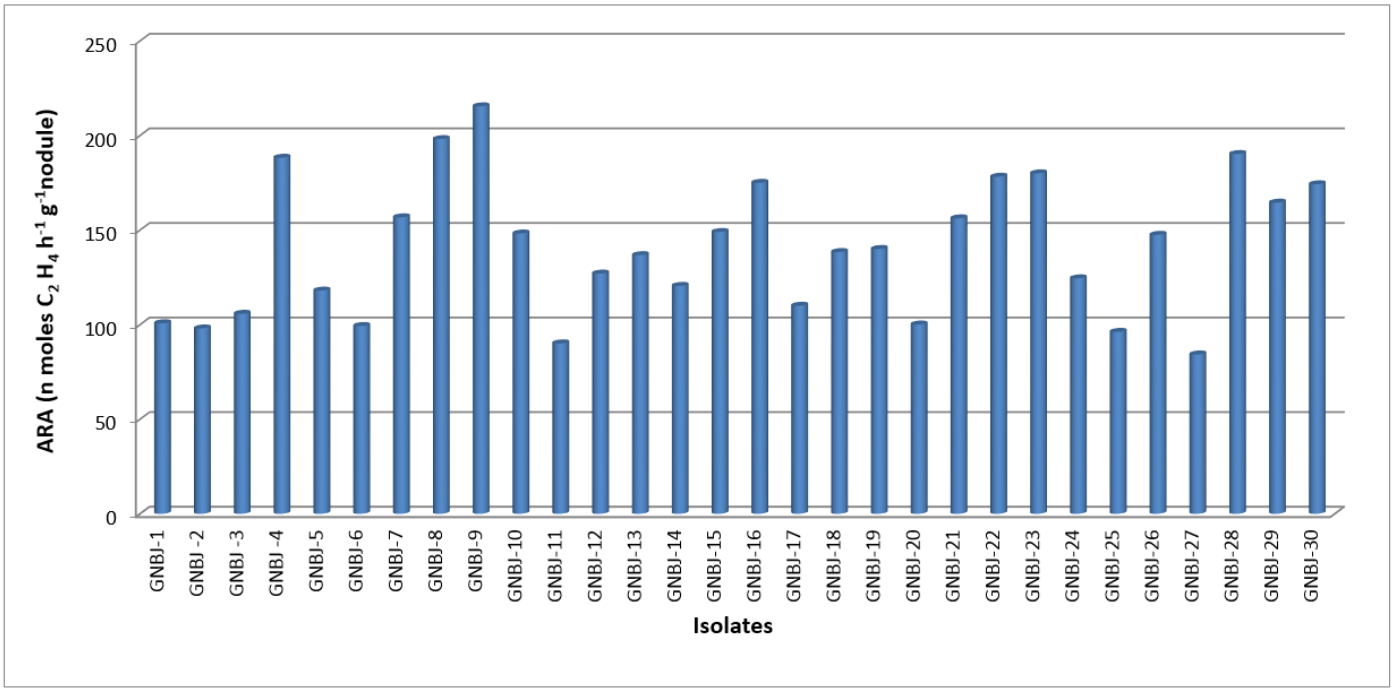

Fig 1: Effect of inoculation of strains of Bradyrhizobia on Nodule ARA Activity of groundnut plants

Table 2: Effect of inoculation of Bradyrhizobia isolates on Total Nitrogen content of Groundnut plants

S.No.	Isolates	Total Nitrogen content (%)
1	GNBJ-1	3.15
2	GNBJ -2	4.00
3	GNBJ -3	3.50
4	GNBJ -4	6.50
5	GNBJ-5	4.65
6	GNBJ-6	4.10
7	GNBJ-7	3.75
8	GNBJ-8	6.85
9	GNBJ-9	7.20
10	GNBJ-10	3.50
11	GNBJ-11	3.98
12	GNBJ-12	4.00
13	GNBJ-13	5.20
14	GNBJ-14	5.90
15	GNBJ-15	4.15
16	GNBJ-16	3.54
17	GNBJ-17	5.25
18	GNBJ-18	3.95
19	GNBJ-19	2.10
20	GNBJ-20	3.50
21	GNBJ-21	3.95
22	GNBJ-22	3.50
23	GNBJ-23	2.55
24	GNBJ-24	4.50
25	GNBJ-25	2.75
26	GNBJ-26	4.94
27	GNBJ-27	2.05
28	GNBJ-28	6.60
29	GNBJ-29	3.60
30	GNBJ-30	2.40
SED		0.27
CD (P=0.05)		0.46

Table 3: Effect of inoculation of Bradyrhizobia isolates on the Leghemoglobin content of Nodules of Groundnut plants

S.No.	Isolates	Leghemoglobin Nodule mg/g		
		15 days	30 days	45 days
1	GNBJ-1	0.640	0.800	0.190
2	GNBJ -2	0.564	0.715	0.420
3	GNBJ -3	0.530	0.710	0.375
4	GNBJ -4	1.420	1.960	1.475
5	GNBJ-5	0.330	0.705	0.460
6	GNBJ-6	0.715	0.805	0.630
7	GNBJ-7	1.320	1.530	1.810
8	GNBJ-8	1.535	1.860	1.640
9	GNBJ-9	2.100	2.225	2.110
10	GNBJ-10	0.790	1.350	0.590
11	GNBJ-11	0.325	0.585	0.400
12	GNBJ-12	0.215	0.681	0.570
13	GNBJ-13	0.520	0.680	0.500
14	GNBJ-14	0.420	0.500	0.430
15	GNBJ-15	0.240	0.470	0.360
16	GNBJ-16	0.185	0.275	0.205
17	GNBJ-17	0.220	0.365	0.210
18	GNBJ-18	0.265	0.425	0.310
19	GNBJ-19	0.175	0.205	0.185
20	GNBJ-20	0.320	0.585	0.465
21	GNBJ-21	0.280	0.310	0.265
22	GNBJ-22	0.183	0.265	0.210
23	GNBJ-23	1.307	1.730	0.185
24	GNBJ-24	0.327	0.510	0.425
25	GNBJ-25	0.410	0.656	0.530
26	GNBJ-26	0.540	0.675	0.620
27	GNBJ-27	0.115	0.220	0.185
28	GNBJ-28	1.190	1.400	1.615
29	GNBJ-29	0.210	0.325	0.200
30	GNBJ-30	0.530	0.685	0.495

The root nodulation pattern of groundnut in the soils of saline areas of Cuddalore district of Tamil Nadu was studied. The physico-chemical properties of groundnut soil samples collected from 30 different locations belong to 3 textural types, *viz*., clay loam, sandy loam and Loamy soil. Thirty isolates were screened for efficiency based on IAA production, EPS production, nodulation, nodule ARA activity, nodule N content and leghaemoglobin content. Among the 30 isolates, GNBJ-9 produced maximum IAA of 7.80 µg ml^{-1}, EPS of 318.30 µg ml^{-1} and nodules of 39.00 $plant^{-1}$, nodule ARA activity of 215.40 n moles C_2H_4 $h^{-1}g^{-1}$ nodule and nodule N content of 7.20 percent. The leghaemologbin content was found to be relatively high 2.11 nodule mg g^{-1} in nodules inoculated with strain GNBJ-9 (Pandeeswari *et al*. (2017)) [9].

(Uyanoz and Karaca (2011) [5], Carmen Lluch *et al.* (2004)) [2] Co-inoculating *Pseudomonas* with *R. galegae* bv. *orientalis* had shown to produce IAA that had contributed to increases in nodule number, shoot and root growth and nitrogen content. Both environmental stress factors (acidic pH, osmatic and matrix stress and carbon limitation) and genetic factors (auxin biosynthesis genes and the mode of expression) were shown to influence the biosynthesis of IAA (Spaepen and Vanderleyden 2011) [12]. Gopal and Prasad (1992) [5] reported that graded levels of potassium increased the laemoglobin content of nodules of horsegram plants raised with *Rhizobium* ailation. The leghaemoglobin content increase was maximum (80.16 % over trol) at 50 kg of K_2O ha^{-1}

The relationship among the three factors was found to be positive and significant in both the plants. Not for general plants growth and development by abolishing symbiotic leghaemoglobin synthesis in nodules of the model legume *Lotus japonicus.* (Subba Rao (1977) [13], Huang *et al.,* (1988) [7]

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