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Screening for efficient strains of *Bradyrhizobium*

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ABSTRACT

Among 157 rhizobial isolates obtained from the root nodules of pigeonpea, mungbean, urdbean, cowpea, groundnut and soybean grown in six different places of Assam, only 15 strains *viz.*, A_1, A_7, B_{14}, B_{45}, B_{52}, C_2, C_{10}, G_7, G_{15}, G_{21}, Gn_2, Gn_3, S_3, S_5 and S_8 were found to be nodulation positive and identified as *Bradyrhizobium*. Among the 15 strains, Gn_3 was found the most efficient strain giving the highest acetylene reduction assay (479.01 mmole $g^{-1}h^{-1}$) and leghaemoglobin content (0.125 mM). On the other hand, strain C_{10} showed the highest efficiency in antibiotic resistance against nine different antibiotics and wide host range infectivity nodulating cowpea, urdbean, mungbean and pigeonpea.

Key words: Antibiotic resistance, Leghaemoglobin, Nitrogenase

Nitrogen fixation in agricultural eco-systems is limited because of the absence of efficient nitrogen fixing microorganisms (1). There are deficiencies of specific rhizobia even in traditional legume-growing areas resulting in poor nodulation which ultimately suggests for inoculation with specific as well as efficient rhizobia (13). The yield response to inoculation of legumes depends on the use of highly effective *Rhizobium* capable to compete for nodulation of their hosts (7). In north eastern region of India, it is often observed that legumes nodulate poorly despite inoculation with *Rhizobium*. It has been reported that strains often fail to establish in new habitat (6). Most of the strains commonly used as inoculants in commercial culture are isolated from soils far distinct from the one present in the region. Therefore, it has become necessary to screen for efficient native rhizobia to improve performance of commercial inoculants recommended for the region. The objective of the present investigation was to identify the efficient *Bradyrhizobium* strains isolated from soils of various parts of Assam.

MATERIALS AND METHODS

Effective symbiosis can be achieved when the nodules are formed by efficient and effective strains of rhizobia. Higher nitrogenase activity, leghaemoglobin content in nodules, broad host range, resistance to various antibiotics, *etc.*, are considered as the criteria for selection of efficient strains of *Rhizobium*. A total of 157 rhizobial isolates were obtained from the root nodules of pigeonpea, mungbean, urdbean, cowpea, groundnut and soybean collected from six different places of Assam *viz.*, Nalbari, Hajo, Guwahati, Tezpur, Majuli and Lakhimpur. Fifteen strains *viz.*, A_1, A_7, B_{14}, B_{45}, B_{52}, C_2, C_{10}, G_7, G_{15}, G_{21}, Gn_2, Gn_3, S_3, S_5 and S_8 were found nodulation positive and identified as *Bradyrhizobium* using cowpea as the test crop.

All the 15 identified strains were evaluated for their efficiency in terms of following parameters with cowpea grown in Leonard jar as described by Somasegaran and Hoben (12). The nitrogen content of the plant top was determined using Kjeldahl method. The nitrogenase activity was determined in terms of ethylene (C_2H_4) produced by the reduction of acetylene using Gas Chromatograph (Chemito) as per procedure described by Hardy *et al.* (8). The acetylene reduction assay (ARA) was correlated with total plant nitrogen and dry weight of plant top. Leghaemoglobin (Lb) was determined following the procedure described by Sadasivam and Manickam (10). The Lb concentration was calculated using following formula:

$$\text{Lb concentration (mM)} = \frac{A_{556} - A_{539} \times 2D}{23.4}$$

where D is the initial dilution.

The calculation is based on the equation $E = 23.4 \times 10^3$ mol^{-1} cm^{-1}

The leghaemoglobin concentration was correlated with ARA and total nitrogen of plant. Resistance to a wide range of antibiotics was tested *in vitro* using antibiotic discs of 10 different antibiotics following procedure as discussed by Barua and Borthakur (3). The cowpea group of *Rhizobium* includes a wide range of legume hosts. The selected strains which could nodulate cowpea as test crop were also inoculated on other legumes like urdbean, mungbean, groundnut and soybean. The nodulation was recorded as positive or negative. The strain which could nodulate more number of legumes was termed as more efficient so far as the broad host range infectivity was concerned.

RESULTS AND DISCUSSION

Different *Rhizobium* strains showed high degrees of variability with respect to nitrogenase activity, leghaemoglobin content, antibiotic resistance and host range infectivity.

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Inoculation of cowpea in Leonard jar with different strains resulted in formation of nodules with wide variation in their weight and accumulation of N in the plant biomass. Nodule

Table 1. Shoot and nodule dry weight, number of nodules and total nitrogen of cowpea grown in Leonard jar

Strains	Shoot dry weight (mg plant^{-1})	Nodule dry weight (mg plant^{-1})	Number of nodules	Total N (mg plant^{-1})
A_1	124	10	15	3.41
A_7	77	19	18	1.23
B_{14}	80	16	24	1.55
B_{45}	110	19	16	1.99
B_{52}	108	37	26	2.00
C_2	66	25	19	1.16
C_{10}	196	29	34	4.25
G_7	161	13	25	4.51
G_{15}	229	45	28	4.03
G_{21}	220	39	24	4.62
Gn_2	234	14	22	7.25
Gn_3	168	7	14	5.44
S_3	196	29	36	3.49
S_5	100	24	33	2.10
S_8	199	25	37	4.42
Control (-Rh-N)	45	0	0	0.56

'-Rh' = Without inoculation with *Rhizobium* '-N' = Without nitrogen

weight varied from 7 to 45 mg/plant and number of nodules from 14 to 37 (Table 1). *Rhizobium* strain Gn_3 showed the highest acetylene reduction assay (ARA) *i.e.*, 479.01 m mole $g^{-1}h^{-1}$ while strain A_7 showed the lowest ARA of 2.00 m mole $g^{-1}h^{-1}$ (Table 2). The correlation analysis between total nitrogen and ARA depicted a highly significant positive correlation

Table 2. Acetylene reduction assay (ARA) of different strains of *Bradyrhizobium*

Strain	ARA (μ mole $g^{-1}h^{-1}$)
A_1	101.95
A_7	2.00
B_{14}	10.82
B_{45}	7.03
B_{52}	7.84
C_2	4.39
C_{10}	23.96
G_7	145.27
G_{15}	6.38
G_{21}	12.40
Gn_2	370.26
Gn_3	479.01
S_3	4.87
S_5	16.64
S_8	48.87

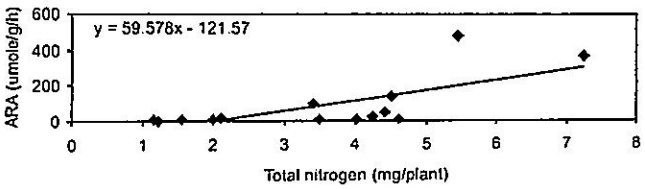

Fig. 1. Total nitrogen vs. acetylene reduction assay (ARA) of cowpea grown in Leonard jar

(r=0.7046) (Fig. 1). Dry weight of shoot was also positively correlated (r=0.3542) with ARA (Fig. 2). Efficiency of legumes on nitrogenase activity was observed by Hardy *et al.* (8) and reported 0.21 m mole min^{-1} mg^{-1} fresh weight of root nodules. The concentrations of leghaemoglobin of the nodules obtained from all the 15 *Bradyrhizobium* strains are shown in Fig. 3. It was observed that the strain Gn_3 produced the highest leghaemoglobin concentration of 0.125 mM in the root nodules of cowpea.

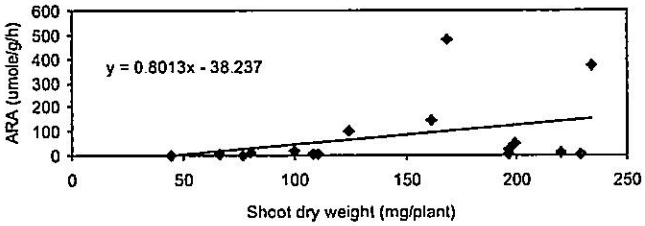

Fig.2. Shoot dry weight vs acetylene reduction assay (ARA) of cowpea grown in Leonard jar

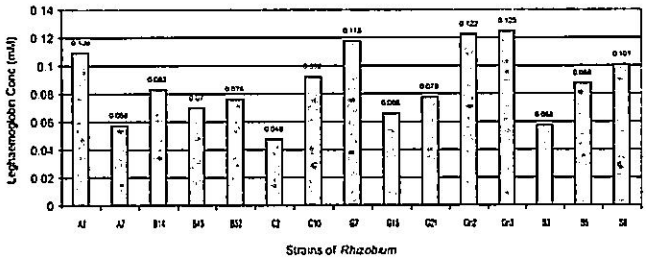

Fig. 3. Leghaemoglobin concentration (mM) of the nodulation positive strains of *Bradyrhizobium*

The results also showed that the leghaemoglobin concentration was positively correlated with nitrogenase activity (r=0.6634) and total nitrogen (r=0.7554) (Figs. 4, 5). The positive correlation of leghaemoglobin with nitrogen fixation (2) and nitrogenase activity in nodules (4) has been reported earlier. The total nitrogen content was improved substantially with inoculation which might be due to higher nitrogen fixation.

It was observed that the *Bradyrhizobium* strain C_{10} came out as the most resistant one against nine different antibiotics *viz.*, streptomycin, kanamycin, penicillin, nalidixic acid, neomycin, tetracycline, ampicillin, erythromycin and chloramphenicol (Table 3). Thus, strain C_{10} was the most

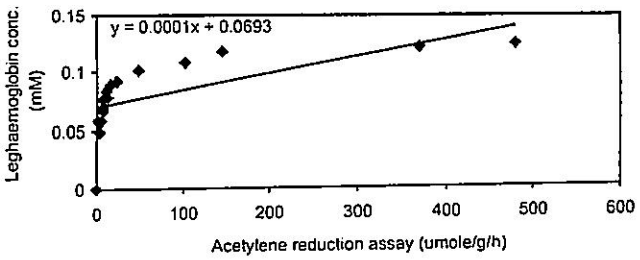

Fig.4. Acetylene reduction assay (ARA) vs. leghaemoglobin concentration of nodules of cowpea grown in Leonard jar

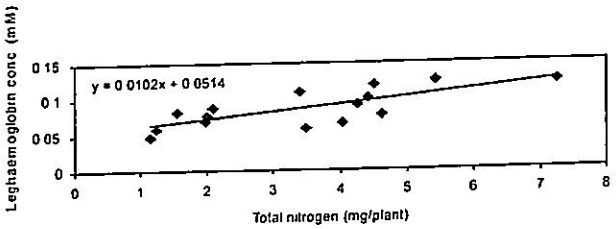

Fig. 5. Total nitrogen vs. leghaemoglobin concentration of cowpea grown in Leonard jar

efficient strain while strain S_5 was the least efficient one being susceptible to most of the antibiotics. The most resistant strain C_{10} was considered to be the best colonizer and competitor in the rhizosphere of legume roots. This had a direct similarity with the view of Scotti *et al.* (11) who opined that the advantage of the use of antibiotic resistant strains of rhizobia was that they could compete efficiently with the antibiotic producing microflora in the soil. The strain C_{10} proved to be the most efficient one nodulating four host plants *viz.*, cowpea, mungbean, urdbean and pigeonpea while strains C_2, G_{15}, S_3 and S_5 could nodulate only two hosts each (Table 4). Dadarwal *et al.* (5) reported that at least 12 crops of agricultural importance were efficiently infected and nodulated by cross inoculating species of *Rhizobium*. Hernandez-Lucas *et al.* (9) working with six divergently related rhizobia reported that three rhizobia were capable of infecting and nodulating 22 legume species. Thus, the present findings of broad host range infectivity towards four legumes supported this view.

Table 3. **Antibiotic sensitivity pattern of different strains of *Bradyrhizobium***

Strains	Antibiotics								
	Streptomycin	Kanamycin	Penicillin	Nalidixic acid	Neomycin	Tetracycline	Ampicillin	Erythromycin	Chloramphenicol
A_1	R	R	R	R	R	S	R	S	R
A_7	R	R	R	R	R	I	R	R	S
B_{14}	R	R	R	R	R	S	R	R	S
B_{45}	R	R	R	R	R	I	R	R	S
B_{52}	I	R	R	I	R	I	R	R	S
C_2	S	R	R	S	R	R	R	R	S
C_{10}	R	R	R	R	R	R	R	R	R
G_7	S	R	R	S	R	S	R	R	S
G_{15}	I	R	R	S	R	S	R	R	S
G_{21}	R	R	R	R	R	R	R	R	S
Gn_2	R	R	R	R	R	I	R	R	R
Gn_3	R	R	S	S	R	R	R	R	R
S_3	S	R	R	R	R	R	R	R	S
S_5	S	S	R	S	R	S	R	R	S
S_8	R	R	R	R	R	S	R	R	S

'R' = Resistant, 'S' = Susceptible and 'I' = Intermediate.

Table 4. **Broad host range infectivity of different *Bradyrhizobium* strains**

Strain	Cowpea	Greengram	Black-gram	Pigeonpea	Groundnut	Soybean
A_1	+	+	-	+	-	-
A_7	+	+	-	+	-	-
B_{14}	+	+	+	-	-	-
B_{45}	+	+	+	-	-	-
B_{52}	+	+	+	-	-	-
C_2	+	-	-	+	-	-
C_{10}	+	+	+	+	-	-
G_7	+	+	+	-	-	-
G_{15}	+	+	-	-	-	-
G_{21}	+	+	-	-	-	+
Gn_2	+	-	-	-	+	+
Gn_3	+	-	-	-	+	+
S_3	+	-	-	-	-	+
S_5	+	-	-	-	-	+
S_8	+	-	+	-	-	+

'+' – Nodules present '-' – Nodules absent

The study revealed that among the 15 strains of *Bradyrhizobium* Gn_3 was found to be the most efficient strain giving the highest acetylene reduction assay (479.01 mmole $g^{-1}h^{-1}$) and leghaemoglobin content (0.125 mM). On the other hand, C_{10} showed the highest efficiency in antibiotic resistance against nine different antibiotics *viz.*, streptomycin, kanamycin, penicillin, nalidixic acid, neomycin, tetracycline, ampicillin, erythromycin and chloramphenicol and wide host range infectivity nodulating cowpea, mungbean, urdbean and pigeonpea.

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