

Potential for Plant Growth Promotion in Groundnut (*Arachis hypogaea* L.) by Inoculation of Native *Rhizobium* Strains

Senthil kumar .R¹, A. Suganya²

Department of Microbiology, PG Extension Centre, Bharathidasan University, Perambalur, Tamil Nadu, India

Abstract: Totally, five different *Rhizobium* strains were isolated and identified from root nodules of *Arachis hypogaea* L. collected from five different fields of Perambalur District, Tamil Nadu, and India. Screening the effect of *Rhizobium* strains on groundnut under pot culture method. Experiments in pot culture yielded promising *Rhizobium* strains introduced groundnuts increasing the plant biomass, nodule number, and yield compared with control. Among the five strains, strain number 3 (RS3) was gave higher shoot length (31.3 cm) and root length (17.7 cm), number of nodules (40) and yield (90.5 g) on 90 days.

Keywords: *Rhizobium*, *Arachis hypogaea* L., Nodules, Plant biomass and Pot culture

1. Introduction

The groundnut is one of the world's most popular and universal legume crops, cultivated in more than 100 countries in all six continents. The geographical classification of groundnut is delineated in six regions: the America, Africa, Asia, New East Asia, Europe and Oceania (Gregory *et al.*, 1980). It is currently grown on 25.2 million ha worldwide with a total production of 35.9 million metric ton (FAO, 2005). Developing countries account for about 97% of the world's groundnut area and about 94% of total production (Freeman *et al.*, 1999). On the global scale, India is a major producer of groundnut with a total production of 8.9 million tons per year. The crop play a significant role in the farmers livelihoods by providing the nutritional security and fetching cash revenue. The Legumes are the third largest family of higher plants with more than 650 genera, 18,000 species and are second in agricultural importance (Doyle *et al.*, 2001).

Nitrogen is an essential nutrient for plant growth and development. Plants usually depend upon combined, or fixed, forms of nitrogen, such as ammonia and nitrate because it is unavailable in its most prevalent form as atmospheric nitrogen. Much of this nitrogen is provided to cropping systems in the form of industrially produced nitrogen fertilizers. Use of these fertilizers has led to worldwide ecological problems as well as affects the human health (Vitousek, 1997).

The process of conversion of nitrogen to a combined form by prokaryotes is referred as biological nitrogen fixation (BNF). It was first discovered by Beijerinck in 1901 (Wagner, 2012). Biological nitrogen fixation (BNF) is the cheapest and environment friendly procedure in which nitrogen fixing micro-organisms, interacting with leguminous plants, fix aerobic nitrogen into soil (Franchet *et al.*, 2009).

Interestingly, some plants (legumes) possess a unique ability to establish symbiotic association with nitrogen-fixing bacteria of the family Rhizobiaceae. *Rhizobium* inoculants

significantly improves yield in many leguminous crops and can minimize the use of synthetic fertilizer which is rather expensive and deteriorates soil properties (Laurette *et al.*, 2015). Legumes are grown on approximately 250 Mha and able to fix about 90 Tg of N₂ per year as result of symbiosis with *Rhizobia* (Kinzig and Socolow 1994).

Bacteria of family Rhizobiaceae are symbiotic and effectively convert atmospheric nitrogen which is utilized by the host. Rhizobiaceae family contains six genera namely *Rhizobium*, *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium*, *Azorhizobium* and *Bradyrhizobium* (Okazaki *et al.*, 2004). Symbiotic nitrogen fixation in legumes takes place in specialized organs called nodules that result from rhizobial infection (Krusell *et al.*, 2005). Peanut (*Arachis hypogaea* L.) a member of family Leguminosae is usually nodulated by rhizobia of genus *Bradyrhizobium* as demonstrated (Van Rossum *et al.*, 1995).

Biofertilizer promotes plant growth and productivity has internationally been accepted as an alternative source of chemical fertilizer. Rhizobacteria effectively colonize plant root and increases plant growth by production of various plant growth hormones, P-solubilizing activity, N₂ fixation and biological control activity (Deshwal *et al.*, 2011). In the present investigation, isolate native *Rhizobium* strains from nodules of peanut and screening the N₂ fixing ability, morphometric studies and yield comparisons on *Arachis hypogaea* L. in controlled condition.

2. Materials and Methods

Collection of nodulated root *Arachis hypogaea* L.

A total of ten nodulated plants were collection from five different farms at Mangalamedu, Eraiyur, Kurumbalur, Ladapuram and Senjeri of Perambalur District, Tamilnadu, India. Healthy ground nut plant were uprooted carefully and those plant possessing healthy nodules with pink colour were selected and brought to the department of microbiology PG Extension Centre, Bharathidasan University, Perambalur to isolation of *Rhizobium*.

Isolation of *Rhizobium* species

Isolation of *Rhizobium* was done using yeast extract mannitol agar (YEMA) as described by Rajendran *et al.* (2008). In this healthy, unbroken, firm and pink nodules were selected for the isolation. They were washed under tap water to remove adhering soil particles, after which they were treated carefully with 70% ethyl alcohol for about one minute and 0.01% HgCl₂ for 20 seconds. They were washed thrice with sterile distilled water under aseptic conditions and crushed with sterile crucible. A suspension was made of the crushed with sterile, plated on YEMA medium containing 1% Congo red dye and incubated at 28±1°C for 24 hours. Growth on YEMA plate was observed after the said the incubation period.

Identification of *Rhizobium* species

Pure culture of the isolates were made and then subjected to Gram reaction negative isolation were further subjected to biochemical tests including catalase, oxidase, Voges-proskauer and indole tests for confirmation. Flagellation test was carried out to test for motility using flagella mordant (loffler's mordant).

Effect of *Rhizobium* strains on *Arachis hypogaea*

In the experiment, five *Rhizobium* strains treated with ground nut and one control set up was maintained each treatment has three replications. The sterilized garden soil was filled in eighteen numbers of clay pots. The healthy seeds of ground nuts were seed treatment with *Rhizobium* strains separately and left for an hour under shade for effective contact, without *Rhizobium* treatment served as control. The treated and untreated seeds were sowed in clay pots separately each pot have 5 seeds. The water was irrigated in pots once in a week; trial pot was protected from insects and animals throughout the cultivation period under controlled condition. The following growth parameters were studied on the 30th and 60th days of growth, 3 plants per treatment were removed to study the morphological growth parameters. Shoot length, root length, number of nodules and yield were studied and recorded. At 90th day (the completion of growth phase), the plants were harvested and yield was measured.

3. Results and Discussion

In the present study root nodulating bacterial strains were isolation from the root nodules of *Arachis hypogaea* L. plants growing Perambalur district. Tamilnadu During this study 5 isolates of *Rhizobium* sp. were recovered from ground nut root.

The colonies of *Rhizobium* sp. isolated from *Arachis hypogaea* L. plants nodules isolated in this study were mucoid, raised with smooth edges of the colony was observed. Similarly, the nitrogen fixing bacteria can be isolated directly from the root nodules of the host plant or from the soil (Geniaux *et al.*, 1993). The cellular morphological analysis, 5 isolates were proved as rod shape and Gram negative. These 5 strains were considered as *Rhizobium* genera (Garrity, 1982). In the study correlated our results all strains were Gram negative and did not absorb red colour when cultured in YEMA containing Congo red. Similarly Shetta *et al.* (2011) mentioned that *Rhizobium* strains failed to absorb Congo red strain in the CRYEMA medium.

The five *Rhizobium* strain were found after screening through a series of various biochemical and sugar fermentative tests. The isolates showed hazy appearance on the motility media and also were positive for catalase tests. The results showed that all the isolates observed mucus production, although some have little mucus. The mucoid production would represent a mechanism involved in the process of adaption and survival of *Rhizobium* in adverse conditions of soil and climate.

The biochemical tests performed on the isolates showed that most were positive for catalase, oxidase, Voges-proskauer and Indole test. Only two *Rhizobium* isolates were negative to oxidase test (Table 1). These findings are in close agreement with Javed and Asghari (2008) who have characterized the *Rhizobium* from soil and root nodules of groundnut with same positive biochemical tests.

Inoculation of *Rhizobium* strains significantly enhanced the ground nut shoot length, root length compared with control on 30, 60 and 90 day after sowing (DAS) in pot culture experiments. *Rhizobium* strain 3 (RS3) applied plants were significantly enhanced the morphometric data's compared with other *Rhizobium* strains and control. The RS3 applied plants were recorded higher shoot length (31.3 cm) and root length (17.7 cm), number of nodules (40) and yield (90.5 g) on 90 DAS. Followed by RS4 and RS2 were moderately enhanced plant growth and yield (Table 2 and Fig.1). Similarly, co-inoculation of *Thiobacillus* with *Rhizobium* increased the shoot, root length and plant biomass on 40 and 80 DAS and *Rhizobium* increased the pod yield by 18% over uninoculated control in pot and field experiments (Aanandham *et al.*, 2007). Co-inoculation of *Sinorhizobium fredii* with PGPR (Garcia *et al.*, 2004) and *Acidithiobacillus* with rhizobia (Stamford *et al.*, 2003b) increased the fresh root and stem weight in Glycine max and shoot dry biomass in yam bean (*Pachyrhizus erosus* L.).

Table 1: Morphological and biochemical characteristics of bacterial isolates from root nodules of *Arachis hypogaea* L.

S.No.	Gram stain	Motility	Shape	Indole	MR	VP	catalase	Oxidase	Citrate Utilization	Suspected Organisms
1	-	+	rod	+	-	+	+	+	-	<i>Rhizobium</i> sp.
2	-	+	rod	+	-	+	+	+	-	<i>Rhizobium</i> sp.
3	-	+	rod	+	-	+	+	-	-	<i>Rhizobium</i> sp.
4	-	+	rod	+	+	+	+	-	-	<i>Rhizobium</i> sp.
5	-	+	rod	+	+	+	+	+	-	<i>Rhizobium</i> sp.

Table 2: Effect of inoculation of *Rhizobium* strains on plant growth, nodulation and yield of ground nut under pot culture conditions

S.NO	Strain	% germinations	Shoot Length			Root Length			Number of nodules	Yield (g)
			30 days	60 days	90 Days	30 days	60 days	90 days		
1.	RS1	95	10.6	21.7	25.8	6.3	12.9	15.4	28	72.3
2.	RS2	95	9.5	20.3	26.1	6.7	13.1	15.8	30	74.2
3.	RS3	100	11.8	27.5	31.3	7.5	14.5	17.7	40	90.5
4.	RS4	100	9.6	22.6	26.5	7.1	13.3	16.1	32	76.3
5.	RS5	100	8.7	19.5	24.2	6.0	12.9	15.2	27	73.0
6.	Control	95	8.0	16.7	21.2	5.8	11.8	14.3	Nil	66

Note RS = *Rhizobium* strain

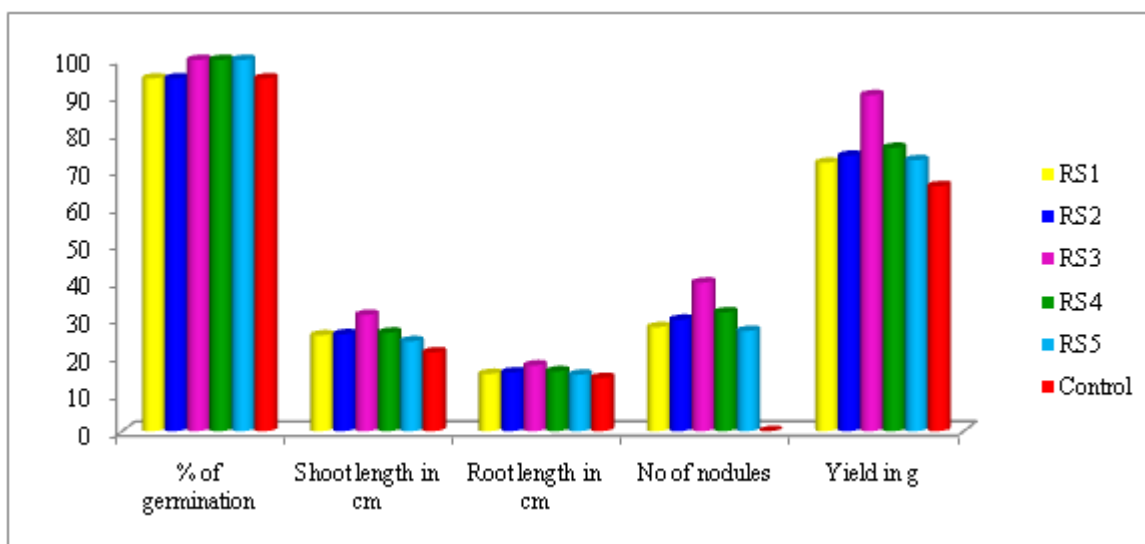


Figure 1: Effect of inoculation of *Rhizobium* strains on plant growth, nodulation and yield of ground nut under pot culture conditions

References

- Anantham R, Sridar R, Nalayini P, Poonguzhli S, Madthaiyan M, Tongmin.S. 2007. Potential for plant growth promotion in groundnut (*Arachis hypogaea* L.) c v. ALR-2 by co-inoculation of sulfur-oxidizing bacteria and *Rhizobium*. *Microbiol. Res.* **169**: 139-153.
- Deshwal, V.K., Vig, K., Amisha, D.M., Yadav, P., Bhattacharya, D. and Verma, M. 2011. Synergistic effects of the inoculation with plant growth-promoting *Rhizobium* and *Pseudomonas* on the performance of *Mucuna*. *Ann. Forestry.* **19** (1): 13-20.
- Doyle JJ., Brenner S and Miller JH. 2001. Leguminosae. *Encyclopedia of Genetics*. Academic, San Diego, USA, 1081-1085.
- FAO, (2005). <http://www.faostatdatabase.org/>
- Franch C, Lindstrom K, Elmerich C 2009. Nitrogen fixing bacteria associated with leguminous and non-leguminous plants. *Plan Soil* **321**: 35-59.
- Freeman, H.A., Nigam, S.N., Kellye, T.G., Ntare, B.R., Subrahmanyam, P, and Boughton, D. 1999. The world of groundnut economy: facts, trends and outlook. ICRI SAT, Patancheru 502324, Andhra Pradesh, India. 52.
- Garcia, J.A.L., Probanza, A., Ramos, B., Barriuso, J., Manero, F.J.G., 2004. Effects of inoculation with plant growth promoting rhizobacteria (PGPRs) and *Sinorhizobium fredii* on the biological nitrogen fixation, nodulation and growth of *Glycine max* cv Osumi. *Plant Soil* **267**: 143-153.
- Garrity A GM 1982. Bergey's manual of systematic bacteriology. *Springer Science and Business Media.* **2**; 325.
- Geniaux E, Laguerre G, Amarger N 1993. Comparison of geographically distant populations of *Rhizobium* isolated from root nodules of *Phaseolus vulgaris*. *Mol. Ecol* **2**; 295-302.
- Gregory, N.C., Krapovickas, A. and Gregory, M.P. 1980. Structural variation, evolution and classification in *Arachis hypogaea* L. *Advances in Legume Science*, Royal Botanical Garden, Kew, UK 469-481.
- Javed.K. and Asghari. B., 2008. Potential alleopathic effects of sunflowers on microorganisms. *Afri. J. Biotech.*, **7**(22):4208-4211.
- Kinzig AP, Socolow RH 1994. Is nitrogen fertilizer use nearing a balance-reply. *Physics Today* **14**: 24-35.
- Krusell L, Krause K, Ott T, Desbrosses G, Krämer U, Sato S, Nakamura Y, Tabata 2005. The Sulfate Transporter SST1 Is Crucial for Symbiotic Nitrogen Fixation in *Lotus japonicas* Root Nodules. *The Plant Cell.* **17**(5): 1625-1636.
- Laurette N. N, Maxemilienn N. B, Henri F, Souleymanou A, Kamdem K, Albert N, Dieudonne N, and François-Xavier E, 2015. "Isolation and Screening of Indigenous Bambara Groundnut (*Vigna subterranea*) Nodulating Bacteria for their Tolerance to Some

- Environmental Stresses,” *American J. of Microbio. Res.* **3**(2): pp. 65-75.
- [15] Okazaki, S., Nukui, N., Sugawara, M. and Minamisawa, K. 2004. Rhizobial strategies to enhance symbiotic Interactions: Rhizobiotoxine and 1-Aminocyclopropane-1-Carboxylate deaminase. *Microb Environ.* **19**(2): 99-111.
- [16] Rajendran .G, Singh .F, Desai A .J. and Archana .G, 2008. “Enhanced growth and nodulation of pigeon pea by co-inoculation of be *Bacillus* strains along with *Rhizobium* sp., *Bioresource Technology*, **99**:4544-4550.
- [17] Shetta, N.D., Al-Shaharani, T.S and Abdel-Aal, M. 2011. Identification and characterization of *Rhizobium* associated with woody legume trees grown under Saudi Arabia condition. *Am. Eurasian J. Agric. Environ. Sci.* **10**(3); 410-418.
- [18] Stamford, N.P., Freitas, A.D.S., Ferraz, D.S., Montenegro, A., Santos, C.E.R.S., 2003b. Nitrogen fixation and growth of cowpea (*Vigna unguiculata*) and yam bean (*Pachyrhizus erosus*) in sodic soil as affected by gypsum and sulphur inoculated with *Thiobacillus* and rhizobial inoculation. *Trop. Grassl.* **37**: 11–19.
- [19] Van Rossum. D., F.P. Schuurnans, M. Gills, A. Muyotcha, H.W. Van Verseveld, A.H. Stouthamer and F.C. Boogerd, 1995. Genetic and phonetic analysis of Bradyrhizobium strains nodulating peanuts (*Arachis hypogaea* L.) roots. *J. Applied and Environ. Microbiol.*, **61**: 1599-609.
- [20] Vitousek.PM. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* **7**:737-750.
- [21] Wagner. S.C. 2012. Biological Nitrogen Fixation. *Nature Edu. Knowl.* **3**(10): 15.