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Development of host specific nodulation by indigenous rhizobia in five agro forestry tree species

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Abstract

Nodulation in legumes provides a major conduit of available nitrogen into the biosphere. 25 geo-specific *rhizobium* isolates in relation to five legume plant varieties were tested with combinations under gnotobiotic conditions for improving nodulation and plant growth through inoculation in field trials for the unraveling and amelioration of sustainable plantations in barren, polluted and agriculture soils. Interestingly, strains from Bhadrachalam forest were found to be highly host specific for *Albizzia lebbeck*, *Sesbania grandiflora* and *Pongamia pinnata* and strains from Jakaram and Kothagudem forests were shown host specific for *Gliricidia maculata* and *Acacia nilotica*, respectively. This method can employ across the country for green revolution.

Key words: Host specificity, nodulation, agro forestry tree species, *Rhizobium*.

Introduction

The degree of host specificity varies tremendously among the rhizobia. Some strains have a very narrow host range while others have a very broad host range. The establishment of symbiotic relationship between legume species and rhizobia is quite complex. Agroforestry has considerable potential, not as the only way to improve agricultural production, but as one important way to enhance and maintain overall productivity of the small upland farm, the agricultural unit that is becoming more prevalent in many parts of the world. Nitrogen fixing tree

species (NFT) possess all the virtues of multipurpose tree species (MPT) and are given top priority in agroforestry. Symbiotic nitrogen fixation in plants occurs in root nodules of legumes and non legumes. The bacterium rhizobium is one of the most studied symbiotic nitrogen-fixing bacteria because it nodulates legumes, which are environmentally significant in soil N fertility management of cultivated lands. The relevance of microbial nitrogen fixation and especially symbiotic fixation to agricultural productivity has sustained interest in this phenomenon for a century (Hungria and Bohrer, 2000). A large number of biotic and abiotic factors can contribute to the inability of an inoculant strain to nodulate under field conditions. Currently establishment of nitrogen fixing bacteria (NFB) in the mycorrhizosphere and manipulation of these microbial associations as a biotechnological tool to enhance plant growth are two important topics of research. Legumes (Fabaceae or Leguminosae) are unique among cultivated plants for their ability to carry out endosymbiotic nitrogen fixation with rhizobial bacteria, a process that takes place in a specialized structure known as the nodule. Legumes belong to one of the two main groups of eurosids, the Fabidae, which includes most species capable of endosymbiotic nitrogen fixation (Wang et al. 2009). These type of studies further requires molecular identification of the microorganisms for the increased specificity of nodulation and that molecular microbial identification can have over culture methodologies (Pavan et al. 2009; 2010), and previous studies suggest that gene identification and characterization can support nodulation and productivity in legumes and non legumes as well.

An efficient Rhizobium is a strain that is able to compete in the field with other indigenous rhizobia for the colonization of the rhizosphere of its homologous legume partner, under various soil physical and chemical conditions. This efficient strain will form many large nitrogen-fixing nodules on the roots of the plant host that will supply, for most legumes, from 70% to 90% of the plant need in nitrogen. Inoculations of *Rhizobium* sp. causes a greater increase in growth and yield and the number of nodules per root system is significantly higher in plants inoculated with *Rhizobium* sp. compared to plants without *Rhizobium* sp. under field condition. In addition to their beneficial N2- fixing activity with legumes, rhizobia can improve plant P nutrition by mobilizing inorganic and organic P. Conjunctive use of *Rhizobium* with Phosphate Solubilizing Bacteria (PSB) revealed synergistic effect on symbiotic parameters and grain yield of mungbean.

Phosphate Solubilizing Bacteria improves the competitive ability and symbiotic effectiveness of inoculated *Rhizobium* sp. in lentil under field conditions (Kumar and Chandra 2008).

Rhizosphere is defined as the soil influenced by roots, bacterial species that carry out functions which promote growth of plants. These bacteria are designated as PGPR (Martı´nez-Viveros et al. 2010). Pseudomonas and Bacillus genera are the most commonly investigated PGPR, and often the dominating bacterial groups in the rhizosphere (Morgan et al. 2005). Bacillus species have been reported to promote the growth of a wide range of plants (Pavan et al. 2013The bacteria stimulate plant-growth even in the presence of several stresses such as drought (Creus et al. 1996).

Root colonizing bacteria (rhizobacteria) that exert beneficial effect on plant development via direct or indirect mechanisms have been defined as plant growth promoting rhizobacteria (PGPR) (Nelson 2004). The concept of plant growth promoting rhizobacteria is now well established for growth promotion. Plant growth promoting rhizobacteria were first defined by Kloepper and Schroth (1978) as the soil bacteria that colonize the roots of plants following inoculation onto seed and they enhance plant growth. The ineffectiveness of PGPR in the field has often attributed to their inability to colonize plant roots (Benizri et al. 2001; Lugtenberg et al. 2001). The synergistic interaction of these pathogens together causes more loss in term of yield than the sum of their individuals to this important pulse crop (Siddiqui and Husain, 1992; Akhtar and Siddiqui 2008a). In addition, microbial activities can be made more efficient by maintaining high bacterial populations in the rhizosphere of a plant throughout the life cycle (Pooran et al. 2002; Bajpai et al. 2002; Cheuk et al. 2003). The seven most efficient N_2-fixing strains can be evaluated for their competitiveness against less effective strains in a pair-wise inoculation experiment and nodule occupancy may determined for the most efficient strain (Hafeez et al. 2001).

Greenhouse and field studies with PGPR strains have demonstrated enhanced nodulation and nitrogen fixation in soybean, lentil, pea, chickpea and common bean (Chanway et al. 1989; Dileep et al. 2001). Plant growth-promoting rhizobacteria (PGPR) are beneficial native soil bacteria that colonize plant roots and result in increased plant growth (Glick, 1995) production of plant growth regulators (De Freitas 2000) and increasing plant water and nutrient uptake (Okon and Labandera-Gonzalez 1994). PGPR can also inhibit soil-borne plant pathogens through antifungal activity (Lifshitz 1987).On the basis of the results drawn from the earlier

investigations of biocontrol agents which perform best under pot trials were selected for the field trial.

The aim of the present research was to investigate the effects Rhizobium of AFT species in field condition. Several attempts were made to screen out and select specific Rhizobium isolates from different forest soils.

1. Materials and Methods :

2.1 Selection of rhizosphere soils.

Undisturbed rhizosphere soils of five agroforestry tree species (*Acacia nilotica, Albizzia lebbeck, Gliricidia maculata, Sesbania grandiflora* and *Pongamia pinnata*) in triplets were collected from five forest soils of Jakaram, Eturunagaram, Mulugu, Bhadrachalam and Kothagudem. The geographical conditions of these areas are similar to that of Mahabubnagar conditions (temperature, pH, moisture).

2.2 Physico-chemical analysis of soil

These rhizosphere soils were not having any previous history of chemical fertilizers, so there was no chance of growth inhibition of natural bio inoculants by the action of chemical fertilizers.

Soil available nitrogen was estimated by alkaline potassium permanganate method (Subbiah and Asija 1956), and available phosphorous was determined after Bray and Kurtz (1945). Potassium determined by flame photo metrically (Jackson 1973).

2.3 Isolation of rhizobia from nodules.

A healthy plant was uprooted with intact soil around the roots. Roots were then carefully washed with a jet of water. Nodules which are pink multilobed and situated on the top root were selected for isolation of *Rhizobium*. Nodule is separated from the root carefully so that piece of root on the side of the nodule remain attached and the nodule itself is not injured. The nodules were thoroughly washed with running tap water placing it a tube with a nylon mesh on one end. The other end of the tube was connected with tap for about five minutes.

Thoroughly washed nodules were transferred to a sterile test tube and immersed in 0.1% $HgCl_2$ solution and 70% ethyl alcohol for 3 min. and one minute respectively. Test tube is shaken periodically in order to remove the adhering air bubbles and to bring the fresh sterilants in contact with the nodules. After three minutes, $HgCl_2$ solution is decanted off and nodule was immersed in alcohol for 3 minutes. After the nodule surface gets sterilized it was washed with sterile water for at least ten times so as to remove the sterilants completely. A few drops of water were left in the test tube containing sterilized and washed nodule. The nodule was crushed with a sterile glass rod with flat end. Care was taken so that test tube may not break during crushing.

Suspension obtained after crushing of nodule was used for isolation of *Rhizobium*. Twenty five ml of Congo red Yeast Extract Mannitol Agar at about 45^{o}C was poured into sterile petridishes. After setting one drop of 10^{-6} dilution suspension was added to the petridishes and spreaded the drop on whole plate. The plates were inverted and incubated at 28 ^{o}C. Small, round colourless, translucent colonies with entire margin are the characters of *Rhizobium*. Colonies which absorbed red colour of Congo red are of common contaminants viz., *Agrobacterium tumifaciens* and *Agrobacterium radiobacter*. Colonies obtained in the plate were transferred to yeast extract mannitol slants for conducting further confirmative tests for *Rhizobium*.

2.4 Following cultural tests were conducted to distinguish rhizobia from contaminations.

2.4.1 Congo red test

Congo red can sometime assist the recognition of rhizobia amongst other kinds of bacteria. In general the rhizobia absorb the dye weekly whereas many of the common soil bacteria take it up strongly.

2.4.2 Growth in alkaline medium

A. radiobacter can be detected by drawing streaks on Hoffer's alkaline medium (pH 11) where *Rhizobium* does not grow, while *A. radiobacter* does. YEMA added with 1 ml/lit of thymol blue (1.6% sol.) is adjusted to pH 11 with (approximately 28 m/N NaOH). On slants, the growth of *Rhizobium* isolates and the change in colour of indicator is observed upto 15 days. If no growth (or) change in colour is observed, it may be *Rhizobium*.

2.4.3 Growth in Glucose - peptone agar

All rhizobia except a few strains of medic rhizobia show no (or) little growth on glucose-peptone agar medium (Glucose 10 g, peptone 20.0 g NaCl - 5.0, Agar - 15.0, Bromo cresol purple 1.0 ml (1.6% alcoholic sol.) pH 7.1) whereas agrobacteria grow well. Observations were taken after 15 days of incubation for growth and change in pH.

2.4.4 Ketolactose test.

Most of the strains of *A. tumifaciens* and *A. radiobacter* have been found to produce 3-Ketolactose in lactose containing medium but not rhizobia. The composition of medium used for this test is same as that of the yeast mannitol agar except mannitol is replaced by lactose (10 g/lit). The medium is poured in plates and on solidification the inoculum is streaked on it. After incubation when sufficient growth is observed, the plates are flooded with Benedict's reagent. Development of yellow ring of cuprous oxide (after 30 min to one hr) around the growth of organism is indicative of *Agrobacterium* contamination.

2.4.5 Nodulation tests

Nodulation tests were conducted by the following methods.

2.4.6 Agar tube method

This method is good to study the nodulation and differentiation of symbiotic effectiveness with plants having small seeds. In this method the plants are wholly enclosed within the glass tube.

2.4.7 Preparation of agar tubes

Sufficient SNA medium (1.0 g $CaHPO_4$, 0.2 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 0.2 g NaCl, 0.1 g $FeCl_3$, 8-15 g Agar, 1 litre Distilled water, (1 ml/litre trace element solution) is added to the medium (15 ml for deep and 20 ml for slope) was put in the tubes (200 mm x 25 mm). The tubes were closed with cotton plugs of uniform depth (20 mm) and moderate compactness. Tubes are autoclaved and set as agar deep tubes or slopes as required.

- **Nitrogen supplied control:** Nitrogen-controls were provided to a final concentration of approximately 70 ppm N (0.05% KNO_3).
- **N-deficient control:** Agar tubes without inoculation are planted with seed or pre-germinated seedlings and put as uninoculated N-deficient control.

2.5 Seed inoculation and plant growth assessment

Sterile soils in triplicates from five forest places were brought to the laboratory and were taken in the pots (Triplicates) and they were sown with the seeds of Agroforestry tree species by inoculating them with different rhizobial strains and then the growth parameters of each plant were assessed.

Undamaged and clean seeds of uniform size, selected to a reasonably uniform size were rinsed with 95% ethanol and immersed for 4 minutes in 0.2% $HgCl_2$ solution. The seeds were then washed thoroughly with at least five changes of sterile water. Seeds known to give germination are sown directly after sterilization and washing, either using two seeds per tube (seedlings later can be thinned to one) or singly, allowing sufficient extra tubes. After 8 weeks of growth, the growth parameters like nodulation efficiency, nodule number, nodule dry weight, plant dry weight and plant height were assessed.

The presences of nodules, their nature at the time of harvesting that are pink because of leg-hemoglobin were accounted as effective. .

The rhizobial strains were isolated and tested for host specificity. All possible combinations of the selected rhizobial isolates were tested under gnotobiotic conditions for improving nodulation to screen the best host specificity.

2.6 Collection of soil samples from problematic sites

Soil samples from problematic sites such as Polluted soils, Barren lands and Agriculture soils were collected in triplets and inoculated with rhizobial strains in order to check the improvement in nodulation and growth of these plants through inoculation in field trials.

2. Results and Discussion.

All the soils under investigation varied slightly in their soil reaction, pH of the different soils was slightly acidic and varied from 5.0 to 5.9. The physico-chemical characterization of the soil samples were done and tabulated (Table: 1)

Table-1: Physico-chemical characteristics of 12 forest areas (NPK in kg/hec)

GPS Location of forest soils collected	**Soil type**	**N**	**P**	**K**	**pH**
Kottagudem Lati 80.64689, Longi 17.54458	Red soil	272.08	104.03	147.00	5.2
Bhadrachalam Lati 80.89359 Longi 17.66879	Red soil clay	263.03	108.11	137.02	5.7
Jakaram Lati 80.32410 Longi 17.58796	Red soil	265.06	105.19	160.65	5.9
Eturnagarm Lati 18.3384786 Longi 80.42698	Red soil	223.01	110.21	150.05	5.3
Mulugu Lati 18.1920214 Longi 79.9457973	Deep black soil	272.73	112.32	160.00	5.2

The results of field trials revealed that the effects of bacterial inoculation on plant growth and symbiotic characteristics given in Table 2, was found to be significant different effects induced by rhizobia on growth parameters of AFT. The uninoculated control and inoculation with Rhizobium alone significantly increased nodule number, nodule dry weight, plant dry weight and plant height host specifically.

A critical perusal of the table-2 reveals that all the isolates under investigation induced the nodulation with AFT species. However, the nodulating efficiency varied with the isolate and the plant. The number of nodules ranged between per plant. A lot of variation is evident with regard to the size, shape and dry weight of the nodules induced by different strains (Figure: 1). Dry weight of the nodules with the different isolated ranged from 0.04 to 0.65. Efficient nodules were produced by BD1 in *Albizzia lebbeck*, BD2 in *Sesbania grandiflora*, BD3 in *Pongamia pinnata*, JK4 in *Gliricidia maculata*, KT5 in *Acacia nilotica* whereas the nodule number produced by other strains was significantly low and showed high host specificity in forming nodules. Nodules produced by MG5 in *Acacia nilotica* and BD2 in *Sesbania grandiflora* were found to be lowest and highest in their dry weights.

Plant height of AFT when inoculated with different rhizobium isolates ranged between 21-60.8 cm. Maximum height 60.8 m was induced by the JK-4 isolate in *Gliricidia*

maculata. The least height of the plant was recorded with the inoculations of MG-3. Increase in the dry weight and nitrogen content of the plant was significant with inoculations of BD-1 in *Albizzia lebbeck*, BD-2 in *Sesbania grandiflora*, BD-3 in *Pongamia pinnata*, JK-4 in *Gliricidia maculata* and KT-5 in *Acacia nilotica*. Maximum nitrogen content recorded was about 3.3 percent and the least recorded was 0.9 in the plants inoculated with host specific strains whose inoculations are equivalent to 30 kg n /ha^{-1}. The uninoculated plants showed significantly low nodulating efficiency among all the plants.

On the whole, it was observed that the strains from Bhadrachalam (BD-1 to BD-3) were found to be highly host specific followed by JK-4 and KT-5 for all the tested legume plants and when inoculated with such strains, improved nodulation and growth of AFT was observed under natural conditions.

Table 2: Screening and isolation of different indigenous rhizobial strains from host-specific agroforestry tree species from forest areas of Telangana State

Name of the plant	Rhizobial strain inoculated	Nodulation			Height of the plant (cm)		Dry weight of the plant (gm)		N Content (%)
		No	Size (mm)	Dry weight (gm)	Shoot	Root	Shoot	Root	
Albizzia lebbeck	**JK1**	29	0.2	0.25	40.3	2429.2	0.95	0.64	2.6
	ET1	19	0.3	0.16	33.2	25.8	0.88	0.57	2.4
	MG1	14	0.2	0.12	24.6	16.8	0.61	0.32	1.2
	BD1	31	0.3	0.28	48.9	38.4	1.02	0.68	2.8
	KT1	30	0.3	0.27	45.6	33.2	1.01	0.67	2.8
Sesbania grandiflora	**JK2**	57	0.5	0.45	48.7	24.8	1.94	0.86	2.8
	ET2	45	0.6	0.39	45.3	2.8	1.63	0.74	2.5
	MG2	25	0.6	0.31	33.4	19.7	0.91	0.49	1
	BD2	74	0.7	0.65	55.4	29.2	2.51	0.95	3.2
	KT2	64	0.6	0.51	51.4	27.1	2.18	0.92	3.1
Pongamia pinnata	**JK3**	36	0.7	0.56	54.4	34.3	2.17	1.37	3
	ET3	24	0.8	0.47	34.8	23.1	1.76	1.02	3.3
	MG3	15	0.5	0.18	21.8	17.1	0.82	0.48	1.4
	BD3	30	0.6	0.51	39.7	29.4	1.95	1.13	2.5
	KT3	27	0.7	0.49	36.4	25.2	1.81	1.06	2.9
Gliricidia maculata	**JK4**	54	0.2	0.22	53.7	35.8	2.04	0.91	2.5
	ET4	42	0.2	0.18	49.2	30.3	1.72	0.86	2.2

	MG4	20	0.2	0.11	36.4	25.1	0.19	0.51	1
	BD4	52	0.2	0.23	60.8	42.5	2.25	1.02	2.8
	KT4	32	0.3	0.12	60.2	40.6	2.12	0.98	2.7
Acacia nilotica	**JK5**	20	0.2	0.11	47.6	28.2	1.05	0.76	2.4
	ET5	12	0.2	0.06	45.3	26.8	0.92	0.66	2
	MG5	7	0.1	0.04	36.9	21.8	0.63	0.28	0.9
	BD5	15	0.2	0.7	49.2	29.1	1.27	0.82	2.5
	KT5	22	0.1	0.11	50.6	29.7	1.39	0.88	2.6
Uninoculated control	**-**	07	0.1	0.03	19.4	13.4	0.28	0.42	0.8

Further, all the possible combinations of the potential rhizobial isolates were tested under field conditions for evaluating the improvement of nodulation and growth in these plants through inoculation in field trials for the unraveling and amelioration of crop production in barren, polluted and agricultural soils. The results showed significantly. The nodulation ability of these isolates was confirmed by inoculation tests. It was observed that the strains played an important role in the growth of plants by showing significant increase in nodulation properties even in barren and polluted soils from the tabulated forms (Figure: 3 to Figure 6 and Table: 3 to Table: 7). The same strain showed host specificity with the same legume tree species hence proving the need of the experimentation and this method can be applied globally for any kind of legume plant or tree species.

Table 3: Effects of Nodulation in AFT by BD-1 in agricultural, barren and polluted soils

	Type of Soil where strains were tested	Nodulation			Height of the plant (cm)		Dry weight of the plant (gm)		N Content (%)
Name of the plant		No	Size (mm)	Dry weight (gm)	Shoot	Root	Shoot	Root	
GM	Agricultural soil	27.00±1.53	0.23±0.013	0.24±0.01	42.33±1.10	31.63±1.29	0.86±0.04	0.58±0.03	2.46±0.06
	Polluted soil	24±1.73	0.22±0.01	0.24±0.01	36.66±1.07	22.03±0.80	0.80±0.07	0.51±0.03	2.4±0.11
	Barren soil	24.33±1.66	0.14±0.01	0.14±0.01	38.56±0.84	26.6±1.25	0.93±0.02	0.71±0.04	2.4±0.20
SG	Agricultural soil	18.66±2.02	0.32±0.01	0.16±0.01	35.5±1.35	27.23±0.83	0.93±0.03	0.66±0.04	2.6±0.11
	Polluted soil	20±1.73	0.24±0.01	0.19±0.01	33.3±1.38	26.53±0.82	0.84±0.05	0.52±0.03	2.1±0.17
	Barren soil	14±1.15	0.24±0.02	0.17±0.02	33.53±1.04	24.36±1.03	0.84±0.05	0.53±0.03	1.76±0.29
PP	Agricultural soil	15.33±0.88	0.25±0.01	0.12±0.01	26.3±0.95	18.16±0.77	0.68±0.04	0.42±0.05	1.53±0.17
	Polluted soil	18.66±1.45	0.26±0.01	0.13±0.01	22.03±1.43	17.6±0.92	0.57±0.06	0.35±0.04	0.8±0.20
	Barren soil	16.66±1.76	0.15±0.01	0.15±0.01	24.6±1.37	16.46±1.70	0.69±0.07	0.25±0.03	0.9±0.20
	Agricultural soil	31.66±1.76	0.33±0.02	0.25±0.01	48.56±1.33	37.9±1.43	1.07±0.04	0.68±0.05	2.76±0.20
AL	Polluted soil	29.33±1.45	0.14±0.01	10.85±10.57	45.7±2.13	39.13±0.92	1.12±0.03	0.82±0.04	1.63±0.26
	Barren soil	33±1.73	0.35±0.01	0.19±0.01	43.06±1.33	37.06±0.87	0.94±0.02	0.63±0.04	2.5±0.23
	Agricultural soil	29.66±1.45	0.32±0.01	0.29±0.01	47.5±1.02	35.93±1.33	1.09±0.04	0.75±0.06	3.13±0.20
	Polluted soil	22±1.73	0.25±0.02	0.18±0.01	45.26±1.74	29.9±1.41	0.97±0.04	0.57±0.04	1.93±0.26
AN	Barren soil	24.66±1.76	0.24±0.01	0.28±0.01	46.7±0.60	31.33±0.67	1.16±0.02	0.58±0.05	2.46±0.17

Values are significant at 0.05

Table 4: Effects of Nodulation in AFT by BD-2 in agricultural, barren and polluted soils

	Type of Soil where strains were tested	Nodulation			Height of the plant (cm)		Dry weight of the plant (gm)		N Content (%)
Name of the plant		No	Size	Dry weight	Shoot	Root	Shoot	Root	
			(mm)	(gm)					
AL	Agricultural soil	56±2.08	0.56±0.17	0.43±0.05	47.73±1.77	25±1.73	1.89±0.02	0.79±0.04	2.46±0.20
	Polluted soil	56±1.15	0.73±0.12	0.56±0.07	47.5±1.32	25.26±1.12	1.90±0.06	0.91±0.04	2.93±0.14
	Barren soil	55.66±2.02	0.4±0.11	0.35±0.05	42.83±1.32	23.96±1.36	1.5±0.09	0.65±0.06	2.633±0.14
PP	Agricultural soil	44.33±2.33	0.6±0.17	0.37±0.049	45.66±2.17	22.46±1.28	1.66±0.10	0.73±0.06	2.43±0.17
	Polluted soil	45±1.73	0.6±0.15	0.36±0.04	45.53±1.94	22±1.32	1.54±0.16	0.78±0.05	2.53±0.14
	Barren soil	43.33±1.76	0.56±0.17	0.37±0.05	46.1±2.11	24.43±1.91	1.80±0.08	0.77±0.04	2.73±0.17
GM	Agricultural soil	25±1.73	0.76±0.08	0.4±0.05	35.33±1.00	20.96±0.69	1.08±0.09	0.67±0.10	1.53±0.31
	Polluted soil	27.66±2.40	0.76±0.20	0.44±0.06	38.2±2.97	21.53±1.56	0.97±0.05	0.58±0.15	1.07±0.04
	Barren soil	26±1.15	0.7±0.11	0.37±0.05	33.96±1.44	19.4±1.09	0.85±0.05	0.63±0.12	0.97±0.05
SG	Agricultural soil	77.66±2.02	0.96±0.17	0.76±0.06	58.56±1.74	31.9±1.43	2.66±0.08	1.05±0.05	3.63±0.29
	Polluted soil	72±1.15	0.5±0.11	0.5±0.03	54±1.53	25.2±1.05	2.44±0.13	0.84±0.06	2.9±0.11
	Barren soil	66.66±1.33	0.56±0.14	0.51±0.06	54.4±2.25	28.76±2.07	2.13±0.08	0.86±0.06	0.28±0.04
AN	Agricultural soil	66.33±1.45	0.73±0.06	0.58±0.03	54.9±1.93	29.5±1.28	2.46±0.20	0.99±0.04	3.73±0.34
	Polluted soil	55.66±2.02	0.433±0.14	0.48±0.05	50.8±1.56	26.1±1.19	2.14±0.09	1±0.05	2.97±0.10
	Barren soil	57±1.15	0.6±0.11	0.56±0.05	53.2±0.72	24.36±1.08	1.9±0.12	1.01±0.0	2.66±0.16

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Values are significant at 0.05

Table 5: Effects of Nodulation in AFT by BD-3 in agricultural, barren and polluted soils

	Type of Soil where strains were tested	Nodulation			Height of the plant (cm)		Dry weight of the plant (gm)		N Content (%)
Name of the plant		No	Size	Dry weight	Shoot	Root	Shoot	Root	
			(mm)	(gm)					
AL	Agricultural soil	32.66 ±1.45	0.73± 0.08	0.57± 0.03	40.86±0.76	29.73 ±0.33	2.01±0.03	1.14 ±0.02	3.5±0.76
	Polluted soil	24.66 ±2.02	0.36± 0.08	0.37± 0.04	36.33±1.32	25.63 ±1.41	1.57±0.10	1.03 ±0.03	1.46±0.29
	Barren soil	25±1.73	0.53± 0.18	0.39± 0.06	38.6±2.01	27.3± 1.78	1.64±0.11	0.97 ±0.05	1.86±0.35
SG	Agricultural soil	24±2.30	0.77± 0.15	0.51± 0.08	34.73±0.86	23.46 ±0.97	1.72±0.11	1.04 ±0.05	2.5±0.23
	Polluted soil	24.66 ±1.45	0.96± 0.13	0.61± 0.12	35.63±1.43	25.93 ±2.14	1.83±0.09	1.13 ±0.06	2.73±0.32
	Barren soil	22±2.08	0.633 ±0.14	0.37± 0.07	32.56±1.51	22±1.47	1.67±0.09	0.96 ±0.06	1.81±0.16
GM	Agricultural soil	18.66 ±2.02	0.81± 0.15	0.463 ±0.24	24.86±1.95	20.5± 2.19	0.94±0.06	0.67 ±0.11	1.75±0.19
	Polluted soil	16.66 ±1.76	0.74± 0.18	0.33± 0.10	24.06±2.22	19.36 ±1.97	1±0.10	0.49 ±0.06	1.34±0.08
	Barren soil	16±1.73	0.56± 0.2	0.32± 0.12	22.56±1.58	18.93 ±0.75	0.91±0.08	0.67 ±0.14	1.33±0.13
PP	Agricultural soil	38.66 ±1.45	0.88± 0.09	0.7±0.11	57.33±1.50	36.16 ±1.01	2.24±0.04	1.37 ±0.08	3.22±0.22
	Polluted soil	37.66 ±2.02	0.86± 0.18	0.73± 0.19	55.36±1.76	34.8± 1.21	2.06±0.04	1.58 ±0.13	3.3±0.26
	Barren soil	33±1.73	0.43± 0.18	0.39± 0.05	53.83±1.37	31.96 ±1.50	1.87±0.09	1.29 ±0.08	2.46±0.23
AN	Agricultural soil	29±1.15	0.88± 0.09	0.69± 0.14	39.433±1.76	29.03 ±2.09	1.94±0.07	1.17 ±0.08	3.05±0.08
	Polluted soil	26.33 ±1.45	0.74± 0.18	0.72± 0.15	38.1±1.96	26±1.34	1.72±0.07	1.24 ±0.13	2.8±0.37

	Barren soil	28±1.15	0.89±0.19	0.56±0.14	37.7±2.45	26.73±2.02	1.84±0.07	1.07±0.04	2.02±0.06

Values are significant at 0.05

Table 6: Effects of Nodulation in AFT by JK-4 in agricultural, barren and polluted soils

	Type of Soil where strains were tested	Nodulation			Height of the plant (cm)		Dry weight of the plant (gm)		N Content (%)
Name of the plant		No	Size (mm)	Dry weight (gm)	Shoot	Root	Shoot	Root	
AL	Agricultural soil	54±1.15	0.44±0.12	0.34±0.07	56.06±1.51	37.6±1.10	2.17±0.06	0.99±0.04	2.93±0.26
	Polluted soil	50.66±1.76	0.28±0.10	0.2±0.03	52.93±1.25	34.6±0.92	1.97±0.06	0.95±0.06	2.26±0.17
	Barren soil	50±1.15	0.21±0.07	0.26±0.03	53.5±1.49	35±0.95	1.91±0.02	0.93±0.03	2.02±0.03
SG	Agricultural soil	42±1.73	0.25±0.074	0.2±0.01	50.66±1.04	31.4±0.63	1.81±0.05	0.91±0.03	2.27±0.04
	Polluted soil	45.33±1.45	0.21±0.05	0.21±0.02	49.533±1.30	32.23±1.29	1.82±0.09	0.84±0.05	2.12±0.06
	Barren soil	41.33±1.45	0.36±0.08	0.29±0.08	45.73±1.64	33.76±1.57	1.78±0.10	0.86±0.06	2.21±0.06
PP	Agricultural soil	24.33±2.60	0.4±0.11	0.23±0.06	39±1.47	27.56±1.28	0.26±0.037	0.7±0.09	1.5±0.26
	Polluted soil	21.33±2.02	0.32±0.16	0.14±0.031	37.4±1.24	26.43±1.12	0.22±0.03	0.54±0.05	0.9±0.10
	Barren soil	19.33±1.45	0.19±0.05	0.14±0.03	36.53±1.05	25.56±1.40	0.18±0.03	0.56±0.09	0.94±0.08
GM	Agricultural soil	54±2.30	0.25±0.08	0.25±0.03	60.86±0.92	42.26±1.13	2.26±0.05	1.09±0.04	3.06±0.17
	Polluted soil	52±1.15	0.25±0.08	0.29±0.08	59±1.35	40.43±1.19	2.25±0.04	1.02±0.03	2.52±0.24
	Barren soil	49±2.30	0.17±0.04	0.26±0.04	60.36±0.66	41.56±0.89	1.95±0.059	1±0.07	2.43±0.03
AN	Agricultural soil	32.33±1.45	0.29±0.04	0.19±0.04	62.93±1.46	42.36±1.21	2.23±0.06	1.07±0.04	3.16±0.29
	Polluted soil	30.33±1.4	0.24±0.0	0.15±0.04	61.83±1.78	41.56±1.0	1.95±0.04	0.94±0.0	2.56±0.17

		5	5			3		8	
	Barren soil	31.66 ±1.4 5	0.41 ±0.1 1	0.23±0. 07	60.36±1.61	39.26 ±2.2 5	2.05±0.04	1.04 ±0.0 5	2.04±0 .07

Values are significant at 0.0

Table 7: Effects of Nodulation in AFT by KT-5 in agricultural, barren and polluted soils

	Type of Soil where strains were tested	**Nodu lation**			**Height of the plant (cm)**		**Dry weight of the plant (gm)**		**N Conte nt (%)**
Name of the plant		No	Size	Dry weigh t	Shoot	Root	Shoot	Root	
			(mm)	(gm)					
AL	Agricultural soil	21±1. 52	0.22± 0.02	0.14± 0.02	48.4±0.61	28.9± 0.47	1.11±0.04	0.82± 0.04	2.53±0. 08
	Polluted soil	20.66 ±1.76	0.26± 0.01	0.15± 0.02	46.86±0.75	26.1± 1.01	1.14±0.05	0.67± 0.06	2.21±0. 07
	Barren soil	19.33 ±1.45	0.2±0 .01	0.15± 0.01	45.3±1.06	26.23 ±1.18	0.98±0.04	0.73± 0.06	2±0.05
SG	Agricultural soil	14.66 ±1.45	0.26± 0.03	0.126 ±0.04	47.03±0.98	28.3± 0.81	1.05±0.06	0.76± 0.05	2.66±0. 33
	Polluted soil	15.33 ±1.45	0.37± 0.03	0.15± 0.05	48.06±1.04	26.36 ±0.86	0.86±0.05	0.74± 0.04	1.99±0. 10
	Barren soil	16.33 ±1.45	0.14± 0.02	0.12± 0.02	45.4±0.98	29.43 ±1.55	0.97±0.05	0.73± 0.06	1.9±0.1 1
PP	Agricultural soil	9.33± 1.45	0.14± 0.02	0.09± 0.02	38.16±0.72	23.53 ±1.04	0.75±0.06	0.35± 0.05	1.04±0. 07
	Polluted soil	9.66± 2.33	0.27± 0.04	0.1±0 .04	37.03±0.66	23.6± 1.81	0.68±0.07	0.34± 0.07	0.98±0. 14
	Barren soil	11.33 ±1.76	0.16± 0.03	1.08± 0.96	39.8±1.47	21.2± 1.83	0.75±0.06	0.35± 0.06	1.73±0. 40
AN	Agricultural soil	23.33 ±0.88	0.14± 0.02	0.14± 0.02	51.56±0.52	31.13 ±0.76	1.43±0.04	0.92± 0.02	2.7±0.0 5
	Polluted soil	20.66 ±1.76	0.16± 0.037	0.14± 0.03	48.23±0.73	31.43 ±1.57	1.41±0.09	0.86± 0.05	2.6±0.1 1
	Barren soil	18.33 ±1.45	0.36± 0.03	0.13± 0.03	50.3±1.18	26.4± 1.13	1.07±0.05	0.81± 0.09	2.8±0.1 7
GM	Agricultural soil	18.33 ±2.02	0.43± 0.14	0.10± 0.02	51.43±1.21	30.73 ±1.05	1.34±0.05	0.92± 0.05	2.83±0. 20
	Polluted soil	19±1. 15	0.46± 0.08	0.08± 0.02	48.26±1.47	30.7± 1.15	1.186±0.03	0.94± 0.05	2.36±0. 26
	Barren soil	16.66 ±1.76	0.46± 0.17	0.54± 0.25	51.5±2.05	28.7± 1.10	1.07±0.05	0.97± 0.049	2.63±0. 17

Values are significant at 0.05

The results indicate that the rhizobial strains are host-specific to the plants collected from different rhizosphere soils. There was a significant improvement in nodulation and growth of these

plants through inoculation in field trials for the unraveling and amelioration of crop production by rhizobium in barren, polluted and agricultural soils.

3. Conclusion and Future directions

This study aimed at the improvement in nodulation and growth of these plants through inoculation in field trials for the unraveling and amelioration of crop production by rhizobium in barren, polluted and agricultural soils. The results indicate that the rhizobial strains are host-specific to the plants collected from different rhizosphere soils. Most of the plant growth promoting characteristics with rhizobium can increase the proportion of seed per pod and productivity in plants, but application of complementary inorganic nitrogen fertilizer in soils with low nitrogen content is needed. Further characterization of this strain may help in providing the interesting results. Our current understanding of the early events in nodulation is based on the identification and characterization of a substantial collection of host plant and bacterial symbionts genes. A sophisticated genetic network controlling the perception and early response to rhizobial NFs is now well established. Approaches targeting events downstream of the early nodulation events will help to provide a more comprehensive view of the relationship between nodule development and systemic regulation of nodulation in legumes. The above method is an easy and cost effective method for the selection of efficient rhizobium bioinoculants for the application to the legume plants in soils of same geographical region and it can be applied globally across latitudes and longitudes.

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Conflict of Interest

The authors declare that they do not have any conflict of interests.

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