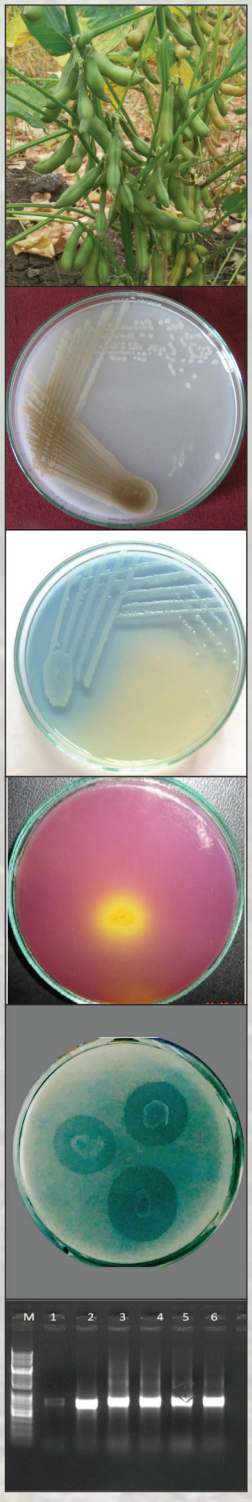
SOIL BIODIVERSITY-BIOFERTILIZERS
Research Progress
2014 - 2016



ALL INDIA NETWORK PROJECT ON SOIL BIODIVERSITY-BIOFERTILIZERS
ICAR-Indian Institute of Soil Science, Bhopal
(Indian Council of Agricultural Research)

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ON

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ICAR- Indian Institute of Soil Science
(Indian Council of Agricultural Research)
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Cover photo: Microbial Diversity, Genomics and Crop Improvement

Back cover: Soil Biodiversity and Biofertilizer Applications in Field.

Inside back cover: Transfer of Technology Trainings and Livelihood Improvement.

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Preface

The All India Network Project on Biofertilizers was initiated by the Indian Council of Agricultural Research in 2004 and then renamed as All India Network Project Soil Biodiversity-Biofertilizers in 2007. The mandate of the project is to enhance the productivity, improve soil health and supplement a part of the chemical fertilizer needs of crops through exploiting the soil biodiversity for use as biofertilizers in diverse cropping systems and agro-ecological zones in India. Improving biofertilizer technology is a priority and there are continuous efforts to extend the biofertilizer applications to disadvantaged areas, including tribal areas and north-eastern hills region. Increasing land degradation and diminishing factor productivity has firmly focused the need for soil health improvement. The UN declaration of the year 2015 as the International Year of Soils was a recognition of the urgent need to redouble our efforts on improvement of soil health. The UN declaration of the year 2016 as the International Year of Pulses is fortuitous since legume crops improve soil health, fix atmospheric nitrogen and improve soil organic matter all of which lead to improved soil health. The focus of the All India Network Project Soil Biodiversity-Biofertilizers and the intensive efforts over last five years on development of genomic tools for soil health assessment, exploration of the genetic diversity of rhizobia to ensure most efficient nodulation and nitrogen fixation by legumes, improvement of soil health and nutrient use efficiency through biofertilizers, improved liquid biofertilizer technology and the dissemination of the technology among tribal farmers leading to livelihood improvement are some of the indicators of the outcome of the project and the successful realization of the mandate of the project during the 12th plan.

I am grateful to Dr. A. K. Sikka, Deputy Director General (NRM), ICAR, New Delhi for his advice on various aspects of the project and to Dr. S.K.Chaudhari, Assistant Director General (Soils), ICAR for his ready help at all stages. I am grateful to Dr. A.K. Patra, Director, IISS, Bhopal for providing the facilities for coordinating the research programmes of the project. I most sincerely thank all the scientists and other staff associated with the project at various universities/Institutes for conducting the researches as per the mandate, for their cooperation in all ways, submitting regular reports and their heads of departments and other administrators for facilitating the smooth conduct of work. I am grateful to Dr. K.Aparna, SRF, IISS, Bhopal for help in finalizing the report for publication.

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Research Highlights

Microbial Diversity for Biofertilizers

More than 2k isolations of rhizobia of 20 major legumes made all over India including 700 from hyper-arid and arid regions of Rajasthan and Haryana; acid soils of Jharkhand, 'Taal' lands of Bihar, soils of Uttarakhand. More than 300+ strains sequenced for 16S rRNA gene. In groundnut, nodulating bacteria other than *Rhizobium* identified.

In Vertisols of central India, population of soybean rhizobia rebounded after monsoon by 13 fold in soybean based crop rotations but only by 1.7 fold in cereal rotations. Regular application of farm yard manure improved the soybean rhizobial numbers by 1.5 fold over chemically fertilized site and 2.5 fold over unfertilized. Slow-growers (genetic homology to *Bradyrhizobium japonicum*) fixed more nitrogen (+15%) than fast growers with homology to *Rhizobium* (*Agrobacterium*) *radiobacter*. The proportion of slow-growers was lower in soybean based rotations (38%) as compared to cereal based rotations (64%).

Rhizobia of arid soils were characterized for stress tolerance and PGPR attributes. High temperature and drought tolerant isolates screened. Inoculation significantly improved the yields of cluster bean, mungbean and pigeonpea under rain-fed conditions. Rhizobia of arid soils showed high genetic diversity.

In lowland rice in Bihar, endophytic rhizobia in 'Desraiya' rice found to have very high genetic homology to rhizobia of the stem and root nodules of the aquatic legume *Aeschynomene* sp. Metagenomic analysis showed `Desariya' rice roots to be rich reservoir of bacterial species (>2k genotypes) belonging to 29 phyla including archaea. The relative abundance of methanogens was about half of that of the methylotrophs.

Several *Rhizobium* isolates of pigeon pea, chickpea and soybean in acid soils analyzed by proteomic tools, which showed unique protein differences amongst acid-soil tolerant isolates. Several genes implicated in imparting adaptation to soil acidity identified.

Potassium solubilizing bacteria isolated from rhizosphere of crops in NEH region identified by 16S rRNA gene sequencing as *Bacillus cereus, Klebsiella variicola* and *Klebsiella* spp.

DAPG-producing fluorescent pseudomonads reduced the incidence of collar and stem rot and improved groundnut pod yields in multi-location field trials.

Nucleus seed inoculum of VA-mycorrhiza was improved by fortifying the carrier with nutrients resulting in halving the rate of biofertilizer application rate required for rice.

Formulations of actinomycetes (strains A10 and A17) along with PGPR (P3, P10 and P25) significantly improved the yields of wheat and that of soybean and chickpea (with *Rhizobium* added) in Vertisols of Madhya Pradesh.

Arthrobacter isolates significantly improved the yields of soybean, rice, maize, chickpea and wheat in Vertisols of Madhya Pradesh.

Conjoint application of PGPR (*Bacillus* spp.) and AMF consortia improved the seedling growth of sweet cherry. Application of PGPR gave highly significant yield improvements in tomato and capsicum in Himachal Pradesh.

Bacillus licheniformis applied by drenching of apple plant basin with liquid inoculum gave impressive yield increase at five locations in Himachal Pradesh.

Frankia strains were isolated from root nodules of *Casuarina equisetifolia* and *Alnus nitida* and characterized.

Integration of biofertilizers with organic manures and chemical fertilizers in INM package gave best performance for crops in Alfisols of Odisha. Seed coating of green gram seeds with gum acacia or sago as sticker improved the yields in acid soil.

Application of biofertilizer consortia (*Azospirillum*, *Azotobacter* and PSB) as seed treatment in Jute decreased the nitrogenous and phosphatic fertilizer requirement by 50% in Assam.

Soil Genomics for Soil Health Assessment

Metagenomic analysis showed that in organically farmed soils there was higher proportion of copiotrophic bacteria. Keystone bacterial species like *Bacillus, Streptomyces, Pseudomonas, Arthrobacter* and *Bradyrhizobium* were relatively higher. The microbial activity in terms of respiration, transcription and translation was greater in organic farming soil.

In long term integrated nutrient management in Alfisol, soils under INM showed significantly higher copies of 16S rRNA gene than those from control, chemically or organically fertilized soils. There was no difference in abundance of Rubisco gene *cbbL* Form I- photosynthetic and chemoautotrophic carbon-fixing gene in soil. However there was significantly higher abundance of chitinase gene in organic amendment soils (OM and INM) than chemically fertilized soils and unfertilized control.

Novel eubacterial isolates *Pontibacter ummariensis* sp. nov., *Luteimonas tolerans* sp. nov., *Tessaracoccus flavus* sp. nov., discovered from an HCH (hexa-chloro cyclohexane) dump site and characterized. *Pseudomonas* sp. RL from same site was sequenced and its gene repertoire was compared with that of 17 reference ecotypes of *Pseudomonas*. Pan-genome analyses of these strains indicated astoundingly diverse metabolic strategies.

Improvement of Biofertilizer Technology

Post-sowing application of liquid biofertilizers in black gram compensated for yields when farmers miss application of soild biofertilizers at sowing. Liquid biofertilizers enhanced the nodulation and growth of pigeonpea even under severe rainfall deficit.

Potassium solubilizing bacteria improved sorghum yields in Andhra Pradesh and also saved 25% K fertilizer.

Mixed microbial consortium for rapid decomposition of agricultural wastes within 2 months released to farmers of Andhra Pradesh.

Diversification of Biofertilizers Usage and Extension

Among zinc solubilizing microbial isolates, *Trichoderma viride* and *Pseudomonas striata* gave best performance in improving yields of soybean, groundnut and cotton, nutrient uptake and soil available zinc in Vertisols of Maharashtra.

Application of microbially enriched compost improved the yields and quality of hot chilli in north-east India. Organic package of practices for rice based on enriched compost and *Azolla* application

with biofertilizers was demonstrated in KVK's in Assam.

In 'taal' lands of Bihar, *Azospirillum* and *Bacillus* application on seeds and post-planting cyanobacteria inoculation significantly improved the yields of direct seeded rice. Co-inoculation of *Rhizobium* and *Bacillus* improved the yields of lentil and urdbean. Bio-nutrient package consisting of cyanobacteria, *Azospirillum* and *Pseudomonas* enriched mycostraw gave significant improvements in rice yields in both resource rich and resource poor farmers.

60 field demonstrations were carried out on soybean, maize, wheat, chickpea, pea, lentil and rice in tribal farmers' fields in Mandla, Chhindwara, Jabalpur, Dindori, and Balaghat districts of Madhya Pradesh with the application of recommended dose of fertilizers along with biofertilizers leading to significant yield gains over farmers' practice.

210 field demonstrations on biofertilizers were carried out among tribal farmers in Kalahandi and Rayagada districts in the state of Odisha growing vegetables, pulses, cereals, oilseeds and fibre crops. There was significant economic benefit with farmers earning Rs.20/- per rupee investment on compost, biofertilizer and lime. The TSP programme besides generating additional income, also helped in generating year round employment for neighbours and thus checking migration of labourers out of the state. Creation of vermicompost pits out of the TSP fund helped preparation of good quality compost throughout the year, kept the rural environment clean and hygienic and helped save at least 25 per cent of the cost incurred on costly chemical fertilizers.

A consortium of biofertilizers consisting of *Azospirillum lipoferum*, *Azotobacter chroococcum* and Plant Growth Promoting Rhizobacteria (PGPR Mix I) were supplied to 125 farmers in ten tribal settlements in Wayanad, Kerala for ginger, pepper and vegetable cultivation. In Attapady, Palghat, Kerala PGPR Mix I was distributed to 600 farmers engaged in the cultivation of vegetables, pulses, banana, sorghum, groundnut, ragi etc. Over 400 farmers and 50 extension officers were trained on biofertilizer usage at both places.

Liquid biofertilizers have become popular and farmers in Andhra Pradesh are saving 20-25% of chemical fertilizers and reporting 10-15 % additional yields in their crops. The demand for liquid biofertilizers by the farmers with drip irrigation facility for the crops like cotton, turmeric, sugarcane, sweet orange and pomegranate has increased in Maharashtra.

Using the microbial strains of AINP on Soil Biodiversity and Biofertilizers, formulations of biofertilizers worth of Rs. 381.7 lakhs were produced at ANGRAU, JNKVV, and MAU representing 77% return on investment in the project during 2014-16.

I. Introduction

Mandate

To enhance the productivity, soil and crop quality and supplement a part of the chemical fertilizer needs of crops through exploiting the soil biodiversity extant, for Biofertilizers in diverse cropping systems and agro-ecological zones in India, improve Biofertilizer technology and extend the Biofertilizer applications to disadvantaged areas.

Objectives

1. To exploit the microbial diversity in various agro-ecologies for biofertilizer applications in diversified systems.
2. To study the impact of soil management practices on microbial functions and soil health.
3. To improve biofertilizer technology to ensure high quality and improved delivery.
4. To diversify biofertilizer research and application in drylands, degraded soils and tribal areas.

Thrust Areas for XII Plan

- Genetic Diversity of Rhizobia
- Soil Genomics for Soil Health Assessment
- Microbial Diversity and Biofertilizers in Eastern India
- Diversification of Biofertilizer Usage

Budget: Rs. 185.0 lakhs (2014-15)
Rs. 309.6 lakhs (2015-16)

II. Projects and sub-projects

1. Microbial diversity exploration in various agro-ecologies for biofertilizer applications in diverse cropping systems

1.1. Genetic diversity of rhizobia of pulses and oilseeds (DGR, IISS)
1.2. Genetic diversity of rhizobia of crop legumes in arid zone soils (HAU)
1.3. Genetic diversity of rhizobia of crop legumes in hyper arid zone soils (MPUAT)
1.4. Rhiziobial diversity in `Taal areas' and rice growing areas in north Bihar (RAU)
1.5. Rhizobial diversity and applications for Hill Legumes (GBPUAT)
1.6. Developing *Rhizobium* inoculant with dual purpose of nitrogen fixation and antagonism against fungal pathogens (IARI)
1.7. Proteomic analysis of diversified *Rhizobium* isolates to identify functionally important proteins (BAU)
1.8. Molecular characterization of Potassium Solubilizing Bacteria (AAU)
1.9. Evaluation of fluorescent pseudomonads for disease suppression in groundnut (DGR)
1.10. Development of multi-functional microbial inoculum for upland rice based cropping system (CRURRS)
1.11. Development of Actinomycetes formulations and testing on field crops (IISS, JNKVV)
1.12. Diversity of *Arthrobacter* in Vertisols and testing on field crops (IISS, JNKVV)
1.13. Development of biofertilizer technologies for selected temperate fruits, vegetables and medicinal plants (YSPUHT)
1.14. Biofertilizers for tropical vegetables in acid soils (OUAT)
1.15. Native diazotrophs for spices (KAU)
1.16. Development of biofertilizers for fibre crops (AAU)

2. Impact assessment of soil management practices on microbial functions and soil health using genomic tools

2.1. Analysis of structural and functional diversity of microorganisms in organic farming practices (UAS)
2.2. Impact of nutrient management on genes involved in carbon sequestration processes in semi-arid tropical soils (TNAU)
2.3. Cultural and metagenomic analysis of pesticide contaminated soils (DU).
2.4. Split-agar assay of antifungal soil microbial metabolites (IISS)

3. Improvement of Biofertilizer technology for high quality and improved delivery

3.1. Refinement and testing of Liquid Biofertilizer Technology (ANGRAU)
3.2. Biofertilizer technology for VA Mycorrhiza (ANGRAU, CRURRS)
3.3. *S*eed coat formulation of bioinoculants for pulses (TNAU)

4. Diversification of Biofertilizer research and application in drylands, degraded soils, tribal areas and NEH region

4.1. Evaluation of zinc solubilising microbial strains for field crops in Vertisols (MAU)
4.2. Evaluation of zinc solubilising microbial strains for rice in NEH region (AAU)
4.3. Exploitation of soil microfauna for sustainable cropping (AAU)
4.4. Development of microbially enriched compost for INM and organic farming in NE India (AAU)
4.5. Demonstrations of improved bionutrient packages for pulses and other crops (RAU)
4.6. Demonstrations on Biofertilizers in tribal areas (OUAT, JNKVV, KAU)
4.7. Other outreach Programmes
4.8. Biofertilizer Production

III. Research Achievements

1. Microbial diversity exploration in various agro-ecologies for biofertilizer applications in diverse cropping systems

1.1. Genetic Diversity of Rhizobia of Pulses and Oilseeds

Studies on the genetic diversity of rhizobia nodulating 20 major legumes in India are under progress. More than 2000 isolations of rhizobia nodulating groundnut, Chickpea, Pigeon pea, Cowpea, Soybean, Black gram, Mungbean, Pea, Lentil, Faba bean, Moth bean, Cluster bean, Lucerne, Berseem, Horse gram, *Sesbania*, Methi etc., have been made from the major growing zones and soil types in Andhra Pradesh, Madhya Pradesh, Gujarat, Rajasthan, Haryana, Uttar Pradesh and Jharkhand. The rhizobia characterized and authenticated by nodulation testing. 16S rRNA gene of 216 nodulating isolates of different crops has been sequenced (~1000 bp). Sequencing of 115 more isolates is under progress.

Diversity of groundnut rhizobia (DGR)

Initial results of 16S rRNA gene sequence of groundnut nodulating bacteria indicated that besides traditional rhizobial genera, others like *Enterobacter cloacae*, *Pantoea dispersa*, *Ochrobactrum* etc. are also found to be nodulating and fixing nitrogen in groundnut (Fig 1). It is likely that due to horizontal and vertical flow of nodulation and nitrogen fixing genes in nature, new genera and species of groundnut rhizobacteria became nodulating and nitrogen fixing. The results are being confirmed further.

Diversity of Soybean Rhizobia in Cropping Systems of Central India (JNKVV, IISS)

Soybean has the highest share of biological nitrogen fixation among cultivated legumes. During early years of the introduction of soybean cultivation in India, the effects of inoculation with slow growing soybean rhizobia were impressive but declined with time due to the naturalization of the introduced strains. Soybean rhizobia are pre-dominantly slow growing but fast growing strains that evolved from natural populations have been reported globally including from several locations in India. The symbiotic effectivity of three slow and three fast-growing rhizobial strains isolated from Vertisols of Central India was evaluated on soybean var. 9752 in sterilized sand microcosms in a green-house. At 40 days growth, there was variation in nodulation parameters and growth of soybean with both types of rhizobia but overall, the slow growers were superior to fast growers with respect to nodule number (56%), nodule dry matter (25%), shoot dry matter (11%) and total nitrogen uptake (22%) (Table 1). The slow-growers (genetic homology to *Bradyrhizobium japonicum*) fixed ~25% more nitrogen than fast growers with homology to *Rhizobium* (*Agrobacterium*) *radiobacter*. The results have very important implications for strain selection during biofertilizer production to maximize biological nitrogen fixation.

Ensuring adequate populations of effective rhizobia in plough layers is essential to ensure optimum nodulation in legumes. To precisely guide the inoculation needs and responses the relative preponderance of slow and fast growing rhizobia in various agro-ecologies was studied in soybean and cereal based cropping systems in Central India. Population of rhizobia was greater in kharif than rabi, particularly at maximum vegetative growth stage of crops. The presence of the host legume stimulated the rhizobial population by 25 fold (average MPN 1262 cells g^{-1} soil in soybean

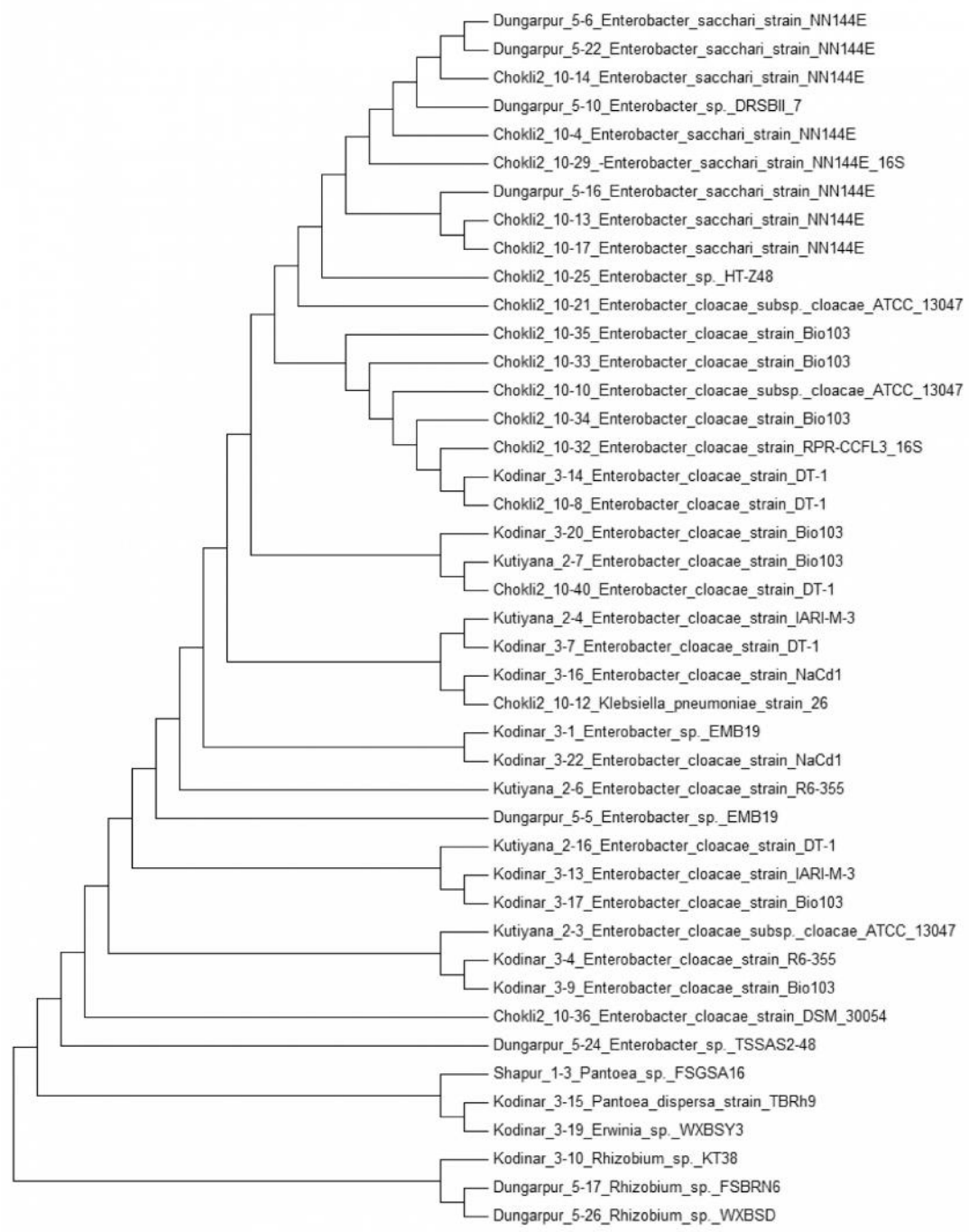


Fig 1. Phylogenetic tree of groundnut rhizobia of Saurashtra, Gujarat.

based cropping sequences as compared to 50 cells g^{-1} in cereal-based sequences). Lowest populations in summer were as low as 9 and 33 cells g^{-1} soil in cereal and soybean rotation soils. Averaged over two years there were 206 rhizobial cells g^{-1} soil in summer at sowing and re-bounded after rainy season at maximum vegetative growth stage to 2743 cells g^{-1} soil (by 13.3 fold) in soybean based rotations. Similarly there were 36 rhizobial cells g^{-1} at sowing and re-bounded after rainy season at maximum vegetative growth stage to 61 cells g^{-1} soil (by 1.7 fold) in cereal based

Table 1. Nodulation and nitrogen fixation by slow and fast growing soybean rhizobia

Rhizobial strain	Nodules no. pl^{-1}	Nodule DM mg pl^{-1}	Shoot DM g pl^{-1}	Shoot N (%)	Total N uptake (mg $plant^{-1}$) (shoot+root+ nodule)	Nitrogen fixation (mg $plant^{-1}$)
Slow-growers: R16	17	65	1.69	1.30	29.1	25.0
R33	25	90	1.81	1.49	34.8	30.7
R34	32	96	1.75	1.44	32.9	28.8
Mean	25	84	1.75	1.41	32.3	28.2
Fast-growers: R8	12	49	1.44	1.15	23.4	19.3
R11	21	83	1.58	1.32	27.3	23.2
R22	16	70	1.7	1.26	28.4	24.3
Mean	16	67	1.57	1.24	26.4	22.3
Control	-	-	1.02	0.31	4.1	-
LSD p=0.05	4.2	8.2	0.09	0.08	2.95	2.95

rotations. In winter the lowest numbers at sowing were 15 and 98 cells g^{-1} soil in cereal and soybean rotation soils. On an average there were 1428 rhizobial cells g^{-1} soil in winter at sowing and did not improve further at maximum vegetative growth stage (1343 cells g^{-1}) in crop rhizosphere following soybean. In rotations following cereals the numbers at sowing averaged 24 cells g^{-1} soil and improved 4.9 fold at vegetative stage in crop rhizosphere. Annual application of farm yard manure in a 40 year long term experimental site in soybean-wheat rotation improved the rhizobial numbers on an average by 1.5 fold over chemical fertilized site and 2.5 fold over unfertilized. Increased crop growth by chemical fertilizers also stimulated rhizobial populations by 1.9 fold over unfertilized soybean-wheat cropping. The proportion of slow-growing soybean bradyrhizobia was lower in soybean based rotations (38%) as compared to cereal based rotations (64%) showing that continued soybean growth leads to higher diversities of rhizobia of the fast growing types. Application of farm yard manure in the long term in soybean-wheat rotation did not change the proportion of slow growers. The slow growers were consistently symbiotically superior and produced greater dry matter and nodule mass (+12%) and biologically fixed nitrogen (+17%) than the fast-growers. Strategies to promote biological nitrogen fixation by soybean should thus include breaking continuous soybean cropping cycles and selecting slow growers as inoculants.

1.2 Genetic Diversity of Rhizobia of Crop Legumes in Arid Zone Soils (HAU)

Rhizobia were isolated from ~100 soil samples from hyper-arid zone of Rajasthan; sandy soils with pH 7.0 to 9.3; organic carbon and EC varied from 0.15 to 0.45% and 0.02 to 0.45 dS m^{-1} respectively. The most probable number (MPN) counts of rhizobia varied from 170-1000 cells g^{-1} soil for different legumes. On the basis of temperature (40 & 45^{o}C) and drought (30 & 40% PEG) tolerance of 202 isolates of mungbean, clusterbean and mothbean, 58 were selected. The isolates obtained from Bikaner and Jaisalmer districts were found to be more stress tolerant as compared to isolates from Churu and Barmer districts. Most of these isolates were P-solubilizers and their solubilization index (P-SI) varied from 1.07 to 5.25. Some of these isolates were good ammonia excretors (36%); however, only 3% produced high amounts of Indole acetic acid (IAA) (> 20 µg ml^{-1}). The bacteriocin production and ACC deaminase activity was also limited to only few isolates. These isolates also showed variable amount of nitrogenase activity in terms of acetylene reduction assay (ARA). The nitrogenase activity of clusterbean rhizobia was on higher side as compared to rhizobia

of mungbean and mothbean. Promising rhizobial isolates were tested for nodulation efficiency and plant growth promotion under stress and non-stressed conditions using Leonard jar assemblies (for testing temperature tolerance) and pots (for drought tolerance using 100, 50 and 25% of field capacity moisture). Most of the isolates of all crops performed better than the respective reference/commercial strains at high temperature (39-45^{o}C) and drought (50-25% FC). The isolates MuJs52b and MuJs72a for mungbean, ClBk 43b, ClJs74b and ClJs87a for clusterbean and MoCh 17b, MoBr 96a and MoBr 99b for mothbean performed better at high temperature (45^{o}C soil temperature) and high drought (25% FC) conditions in terms of nodulation efficiency and plant growth parameters as compared to their respective reference strains and uninoculated control. Genetic diversity and molecular characterization of these isolates on the basis of 16S rRNA gene sequencing is under progress.

Sixteen out of 130 mungbean rhizobial isolates, obtained from South-West Haryana and Rajasthan state were found to have multi-trait characters like P-solubilization, IAA production, nitrogenase activity, ammonia excretion, ACC utilization, and siderophore and bacteriocin production. Two mungbean rhizobial isolates, MR54 and MH8b2, isolated from Rewari and Hisar districts, respectively were selected as more efficient isolates than commercial strain, 703 on the basis of nodulation efficiency and plant growth parameters, when inoculated either individually or in co-inoculation with PSB in pots.

Field evaluation of arid zone rhizobia

Abiotic stress tolerant clusterbean, mungbean and pigeon pea rhizobia obtained from arid and semi-arid zones were tested for their efficacy under rain-fed conditions at CCS HAU farms and RRS, Bawal (Fig 2). Most of these isolates showed significant increase in nodulation efficiency and seed yield as compared to uninoculated control and commercial strain. Out of 8 promising clusterbean rhizobia, six isolates (GB14c, GB32a, GB32c, GH1a, GH2b and GM16b,) showed significant increase in seed yield of clusterbean variety HG2-20, which varied from 1.35 - 1.47 Mg ha^{-1} as compared to control (1.2 Mg ha^{-1}) at recommended dose of fertilizer and commercial strain GSS (1.29 Mg ha^{-1}). Likewise 10 pigeon pea and 5 multi-trait mungbean rhizobia were also checked for their efficacy in pigeon pea variety Paras and mungbean variety MH 421 with application of recommended doses of fertilizers in micro and drought plots at CCS HAU farm. All these isolates showed better nodulation efficiency and plant growth. The isolates MR63, MB17a and MR54 were selected as promising multi-trait mungbean rhizobia, which showed 9.1, 7.5 and 4.8% increase in seed yield as compared to control (517 kg ha^{-1}). The inoculation of the isolate MR63 resulted in highest seed (564 kg ha^{-1}) and straw (1490 kg ha^{-1}) yield. In case of pigeon pea, the isolate PPM37D, PPM33B, PPB25A and PPH10B were selected as most efficient isolates, which resulted in 7.1, 5.9, 5.4 and 4.7% increase in seed yield, respectively as compared to control. Inoculation of these isolates also resulted in increased N and P uptake after harvest of crop.

Fig 2. General view of experiments on inoculation of rhizobial isolates of clusterbean (a) at CCS HAU, RRS Bawal and pigeon pea (b) at Hisar farm.

1.3 Genetic diversity of Rhizobia of leguminous crops in arid zone soils of Rajasthan **(MPUAT)**

About 60% of the Rajasthan state falls under hot arid desert region. The diversity of effective nodulating strains of rhizobia from different agroecological regions of the state was explored for selection of efficient, stress tolerant rhizobial strains with multiple plant growth promoting activities that can be deployed for inoculation of legumes to improve crop productivity and soil fertility. About 400 rhizobial stains were isolated, purified and preserved from different areas of Rajasthan from clusterbean (5 no. strains), green gram (12), soybean (36), black gram (19), cowpea (14), groundnut (37), chickpea (112), methi (52), berseem (16), lucerne (25), pigeonpea (3), pea (25) and sunnhemp (41).

The rhizobial cultures obtained from chick pea, methi and groundnut were screened for plant growth promoting traits and for tolerance to high temperature, salt, pH and drought (using polyethylene glycol). Among 51 strains from chickpea, 30 could grow at 40°C and 17 at 45°C. Among 38 strains from Methi, 24 could grow at 40°C and only 6 strains could grow at 45°C. Six strains from Chickpea, 3 strains from Methi and 5 strains from groundnut also showed growth at 30% PEG. Four strains (SBD-213, SBD-224, SBD-225 and SBD-235) from groundnut also exhibited growth at 40% PEG. Out of 51 strains of chickpea, 9 strains (SBD- 61, SBD-63, SBD-65, SBD-69, SBD-72, SBD-74, SBD-85, SBD-89, and SBD-103) were found to nodulate under *in vitro* controlled conditions. Out of 30 strains of groundnut, 14 strains (SBD-210, SBD-211, SBD-13, SBD-214, SBD-215, SBD-222, SBD-223, SBD-227, SBD-228, SBD-230, SBD-232, SBD-233, SBD-234 and SBD-235) were found to nodulate under *in vitro* controlled conditions. This showed the presence of a number of non-nodulating endophytes inside root nodules.

The 30 rhizobial strains of groundnut isolated from Udaipur, Jaipur, Ajmer, Dausa, Nagaur and Bhilwara regions were characterized for PGPR activities. All of them produced ammonia and siderophores, 9 were phosphate solubilizers (PSI ranging from 2.1 to 2.8) and 16 were IAA producers. 38 rhizobial strains from root nodules of Sunhemp were collected from Ajmer regions and characterized; 22 strains were positive for ammonia production. Of 52 rhizobial cultures from root nodules of cluster bean, green gram, soybean, cowpea, black gram and groundnut, 13 were ammonia producers, 14 were phosphate solubilizers, 21 were moderate to high IAA producers and 17 were siderophore producing strains.

The rhizobial cultures obtained from chickpea (48), methi (33) and groundnut (30) were subject to molecular analysis. The genomic DNA was isolated from these rhizobial strains and tested for *nod*D and *nif*H gene amplification for authentication of rhizobia. Amplification of 16S rRNA gene with 27F and 1378R primers and ARDRA was performed using the digest of 16S rRNA amplicons with *Alu*I, *Taq*I, *Hinf*I and *Hae*III. Dendrogram of groundnut strains obtained by UPGMA method differentiated the 30 strains into 2 major clusters and 4 minor clusters with a coefficient ranging from 0.22 to 0.93 (Fig 3). Chickpea strains showed significant molecular diversity and the dendrogram obtained differentiated 48 strains into 2 major clusters and six minor clusters, respectively with a coefficient ranging from 0.15 to 0.95 (Fig 3). Dendrogram obtained from Methi strains differentiated the 33 strains into 6 major clusters with a coefficient ranging from 0.31 to 0.85 (Fig 4). According to Jaccard's similarity coefficient, genetic similarity among different rhizobial strains of chickpea, methi and groundnut isolated from the Rajasthan revealed very high genetic diversity.

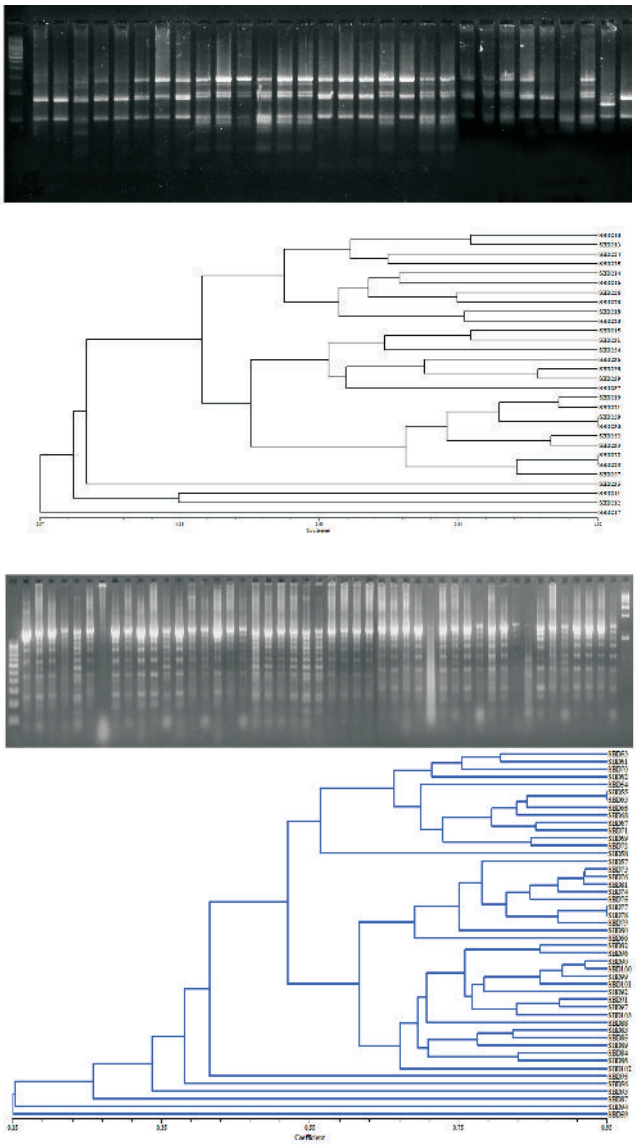


Fig 3. ARDRA pattern of 16S rRNA gene sequences and dendrogram depicting clustering of rhizobial isolates of groundnut (Top-30 isolates) and chickpea (Bottom-48 isolates).

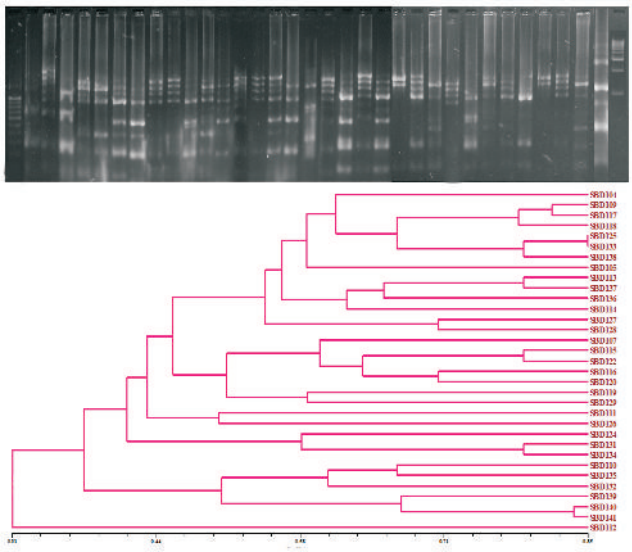


Fig 4. ARDRA pattern of 16S rRNA gene sequences and dendrogram depicting clustering of rhizobial isolates of Methi (33 isolates).

Isolation of Azotobacter, PSB, KMB and ZSM from arid zone soils

A total of 90 soil samples were collected from rhizosphere of various crops grown in kharif and rabi season across various parts of Rajasthan during 2015-16 and isolations were made for *Azotobacter* (49 strains), K solubilizing bacteria (25 no., Phosphate solubilizing bacteria (14 no.) and zinc solubilizing bacteria (39 no.).

1.4. Rhiziobial diversity in `Taal areas' and rice growing areas in north Bihar (RAU)

Rice microbiome

In `chaur' lands of Bihar the aquatic root/stem nodulating legume *Aeschynomene* (Fig 5) grows spontaneously in close proximity to submerged rice. The water submergence varies from 0.3 to 1.5m and rice produces numerous nodal roots. Since the two plants have grown in association for long, rhizobia and other endo-symbionts may be occurring in `Desariya' rice that could be contributing to nitrogen fixation and growth promotion. Investigations were made to characterize the rice microbiome using metagenomic approach at some sites never fertilized with chemical fertilizers.

Fig 5. Root and stem nodules of the aquatic legume *Aeschynomene indica*

Plants were collected from a lowland rice field at Baurgauw (Hasanpur) in Samastipur district, Bihar. DNA was isolated from sterilized roots by modified CTAB method and purified on 1% agarose gel. The genomic DNA was used to amplify the 16S rRNA gene (Fig 6) using 8F and 805R primers which was then sequenced to identify the endophytic bacteria from the NCBI data base.

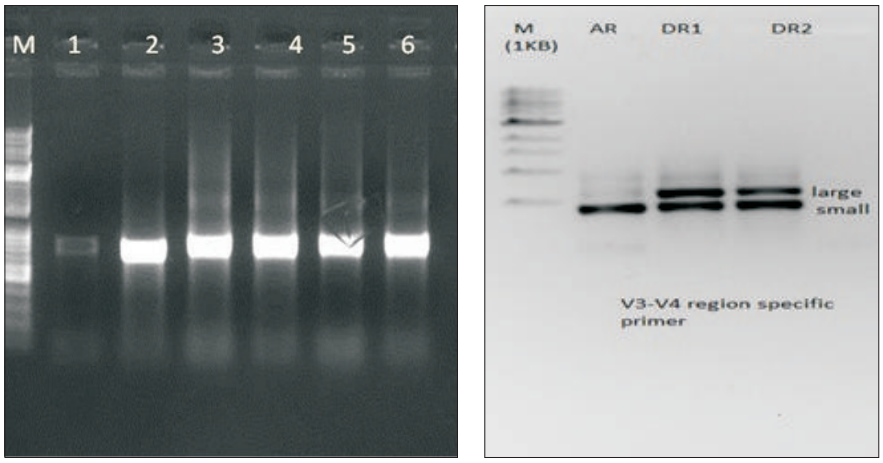


Fig 6. 16S rRNA gene amplicon (~650bps) on 1% agarose gel (left), amplification of v3-v4 region (right).

The 16S rRNA gene of some *Aeschynomene* root and stem nodule endophytes had 99% and 100% similarity to *Bradyrhizobium* sp. IRBG 230. Due to overlap of sequences, four sets of primers were used to target the *Bradyrhizobium* 16S rRNA (ITS region), *fix, nif* and V3-V4 region in order to detect the bradyrhizobia in Desariya rice roots. No bands were detected with *nif* or *fix* gene specific primer but the bands were quite distinct with v3-v4 region specific primer in primary PCR (Fig 6). However, the band was also detected with 16S rRNA (ITS) region which was quite distinct only after secondary PCR. The PCR product of v3-v4 region specific amplification was subjected to Sanger sequencing. Blast results in NCBI data base are shown in Table 2.

Table 2. BlastN result of amplified v3-v4 region of 16S rRNA gene

Sample Names	Sequence length	BlastN hit	Homology
Aeschynomene root nodule	299bp	*Bradyrhizobium* sp. WBOS16 16S ribosomal RNA gene, partial sequence	97%
Desariya rice root in *Aeschynomene* ecosystem DR1	216bp	Uncultured *Bradyrhizobium* sp. clone VPK5W1u01 16S ribosomal RNA gene, partial sequence	97%
Desariya rice root DR2	516bp	*Oryza sativa* japonica Group cDNA, clone: J090063J18, full insert sequence	98%

A clonal library of 16S rRNA gene of 'desariya' rice rhizospheric roots was constructed (96 clones) and sequenced. Information on putative Archaeal/Bacterial endophytes of desariya rice from Desariya rice – *Aeschynomene* ecosystem through metagenome analysis revealed that desariya rice roots are a rich reservoir of microbial community. They showed 2168 types of different bacterial

species belonging to 29 phyla and 44 types of different archaeal species belonging to the phylum crenarchaeota and euryarchaeota. Distribution of putative endophytes, based on rRNA genes indicated that members of proteobacteria dominated the endophytic community (44%), followed by Firmicutes (18%), Actinobacteria (17%), Bacteriodetes (7%), Cyanobacteria (2.5%) and Archaea (2%). Within the phylum Proteobacteria, predominant endophytes were *Rhizobium* groups (23), *Sphingomonas* (22), *Azospirillum* (10), *Burkholderia* (9), *Pseudomonas* (25) and *Desulfovibrio* (28). Similarly the phylum Firmicutes was dominated by *Clostridium* (63) and *Bacillus* (41), Bacteriodetes by *Prevotella* (22) and *Flavobacterium* (14), Actinobacteria by *Streptomyces* (39), Cyanobacteria by *Nostoc* (6) and Archaea by methanogens (24). Among proteobacteria, maximum number of genera (105) were recorded in gammaproteobacteria followed by alphaproteobacteria having 90 genera (Fig 7). Such microbial profiling of rice will facilitate the manipulation of the ecosystem to engineer the rhizosphere to improve the benefits from microbiota for agronomic and ecological benefits.

Rice cultivation is the major source of global methane emissions due to anaerobic conditions. Microbes producing CH_4 were represented by 24 different species of 12 genera and CH_4 utilising microbes were represented by 44 different species of 13 genera. The relative abundance of methanogens was thus almost half to that of the methylotrophs. The complete complement of microorganisms involved in methane cycling were thus present in rice microbiome and it is hypothesized that microbes producing or using CH_4 might form consortia within the rice root system.

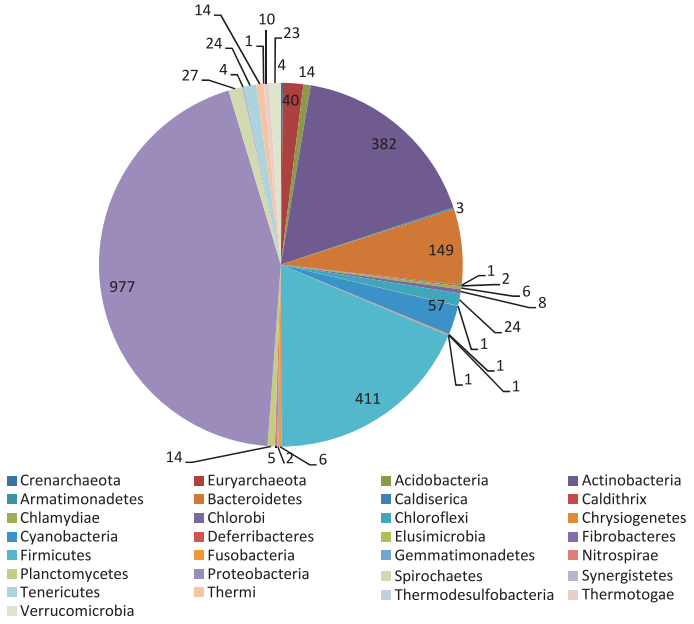


Fig 7. Relative proportion of eubacterial and archaeal phyla in `Desariya' rice root microbiome.

Rhizobial diversity in Tal land

The 'Tal' lands of Bihar are shaped like a saucer and are spread over almost 8 districts, Patna, Nalanda, Munger, Lakhisarai, Shaikhpura, Bhagalpur, Begusarai and Khagaria. Ganga and other rivers of the plateau area of Bihar have their inlet into the Tal land area of about 13,000 sq. km. The Tal land gets filled up with rainwater and floodwater and remains submerged till the last week of September and early October. Thereafter the water begins receding from the second week of October and crops are grown only during the Rabi season. The soils are heavy clay, poor in organic

matter and of the nearly 1.5 lakh ha cultivable area, in majority (75%) of the area, pulses like gram and lentil are grown and this area is therefore considered the pulse basket of Bihar. Forty eight *Rhizobium* isolates were isolated from lentil, pea, lathyrus, chickpea, and bakla; of these thirty nine were fast growers. Utilization of carbon sources by the strains was studied to understand the differences among rhizobia. After screening for nodulation efficiency, twenty three strains of *Rhizobium* were characterized and preserved for production of biofertilizers.

1.5. Rhizobial diversity and applications for Hill Legumes (GBPUAT)

Nodulation of French bean and vegetable pea was evaluated in soils of Uttarakhand drawn from seven districts located at altitudes ranging from 500-4000m above MSL. The nodulation status was ascertained by growing the surface sterilized seeds of respective crop for 45 days in thermocol (polystyrene) containers filled with soils in net house. In French bean, 40% of the soils showed nodulation (Table 3) whereas it was 35% in vegetable pea (Table 4). There was no nodulation of French bean in soils of district Nainital and no nodulation of vegetable pea in soils of district Pithoragarh. However 25-75% and 17-75% of soils from other districts supported the nodulation in French bean and vegetable pea respectively. Meager number of nodules was formed in majority of the soils except few soils of the District Kotdwar in case of French bean and Bageshwar in vegetable pea.

Table 3. Status of natural nodulation in French bean grown in soils of different districts of Uttarakhand

District	Soil samples	Nodulation positive	No nodulation	% of soil samples showing nodulation
Kotdwar (500-1700 m)	21	12	9	57
Bageshwar (950-1300m)	12	4	8	25
Almora (1000-1500 m)	12	3	9	25
Nainital (1500-2000m)	6	0	6	0
Pithoragarh (1500-2000m)	10	3	7	30
Dehradun (>2000m; Chakrata)	4	3	1	75
Chamoli (3500-4000 m)	4	3	1	75
Total	69	21	41	41

Table 4. Status of natural nodulation in vegetable pea grown in soils of different districts of Uttarakhand

District	Soil samples	Nodulation positive	No nodulation	% of soil samples showing nodulation
Kotdwar (500-1700 m)	21	8	13	38
Bageshwar (950-1300m)	12	7	5	58
Almora (1000-1500 m)	12	2	10	17
Nainital (1500-2000m)	6	2	4	33
Pithoragarh (1500-2000m)	10	0	10	0
Dehradun (>2000m; Chakrata)	4	2	2	50
Chamoli (3500-4000 m)	4	3	1	75
Total	69	24	45	35

About 30 rhizobial isolates were obtained from nodules of French bean grown in the above soils. Similarly 25 rhizobial isolates have also been obtained from the nodules of vegetable pea collected from the farmers' field of District Udhamsingh Nagar and Nainital. The chracterization of the isolates is under progress.

1.6 Developing Rhizobium inoculant with dual purpose of nitrogen fixation and antagonism against fungal pathogens (IARI)

The basic tenet of *Rhizobium* inoculation is to improve symbiotic nitrogen fixation in legumes but could also be utilized to control various soil-borne plant pathogenic fungi. Fungal pathogens of the genera *Fusarium, Rhizoctonia* and *Macrophomina* are reported to be controlled by *Rhizobium leguminosarum*, *Sinorhizobium meliloti* and *Bradyrhizobium japonicum*. Earlier ten rhizobial isolates specific to chickpea were reported to be antagonistic against *Fusarium oxysporum*, *Sclerotium rolfsii, Botrytis cinerea* and *Macrophomina phaseolina*. Among them isolate number A13 was found to be most efficient against all the four pathogenic fungi followed by isolates A15 and A10.

All the chickpea-*Rhizobium* strains exhibiting antifungal activity were screened for production of ammonia, HCN, siderophore and antibiotics. Only two isolates specific to chickpea A3 and A16 were found positive for ammonia production. Among all the isolates only CR18 was found positive for HCN production whereas none of the isolates showed production of siderophores. Isolate A-13 was able to solubilise phosphate. Isolates A10, A13, A15, CR18 and CR24 were found positive for IAA production. Among them A15 showed highest IAA production (48 µg mL^{-1}) followed by A13, CR24 and CR9. In a pot experiment on inoculation of chickpea, *Rhizobium* isolates A10 and A3 yielded maximum root and shoot biomass respectively (Table 5) whereas inoculation of CR9 recorded highest nodule number and weight. Maximum ARA value was recorded for treatment with isolate A3 (Table 5).

Table 5. Growth and nodulation parameters of chickpea as influenced by *Rhizobium* inoculation

Treatment	Dry weight (g $plant^{-1}$)			Nodule no. $plant^{-1}$	ARA (µM ethylene produced h^{-1} g^{-1} fw nodule)
	Root	Shoot	Nodule		
Control	0.22	1.38	0.00	0	
A3	0.35	1.96	0.19	11	2.36
A10	0.37	1.86	0.22	14	2.14
A13	0.28	1.46	0.13	08	1.16
A15	0.26	1.52	0.16	12	1.21
A16	0.31	1.74	0.18	11	0.98
CR9	0.30	1.76	0.26	16	1.56
CR14	0.30	1.69	0.24	09	1.66
CR18	0.28	1.64	0.16	10	0.98
CR20	0.33	1.67	0.22	12	1.23
CR24	0.35	1.82	0.21	14	2.05
LSD (p=0.05)	0.07	0.22	0.06	03	0.44

In a field trial at IARI farm the performance of the ten rhizobial strains was evaluated on chickpea along with reference strain F75. After 45 days, all the isolates showed increased shoot, root, nodule fresh and dry weight except isolate CR9 as compared to control. Isolate A13 showed highest shoot biomass followed by A15 and CR24, whereas isolate CR24 showed highest root biomass followed by A13 and A15. Isolates A3, A13, A15 and CR24 were statistically at par and recorded higher nodule biomass as compared to other treatments. Inoculation of A 13 resulted in highest grain yield (1017 kg ha^{-1}) followed by CR24 (963 kg ha^{-1}). Based on the field performance, two rhizobial isolates A13 and CR24 were selected for further evaluation.

***Rhizobium* based biofilmed formulation as biocontrol agent for chickpea**

Rhizobium based biofilms using *Trichoderma viride* as fungal matrix were developed and tested using isolates A13 and CR 24. In a pot experiment chickpea (var. JG- 62) seeds were inoculated with selected *Rhizobium* or *Rhizobium* based biofilms. Plants were challenged with two doses (15 g and 30g pot^{-1}) of virulent pathogen (Wilt complex: *Fusarium oxysporum* f. sp. *ciceri*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*). Among the treatments, single inoculation of isolate CR 24 performed the best and recorded highest root and shoot biomass as compared to other treatments. Control treatment challenged with pathogen but not inoculated with *Rhizobium* or *Rhizobium* based biofilms showed stunted growth but wilt symptoms were not observed. HPLC analysis of shoot extracts showed that concentration of glucose and xylose was higher in inoculation treatments as compared to control. Rhizobial isolate CR24 showed higher accumulation although when fungal dose was increased, isolate A13 showed best antifungal activity. Plants not challenged with pathogen showed least accumulation of sugars. Both the *Rhizobium* isolates and their biofilms showed higher accumulation of organic acids like malic, succinic, formic and propionic acid in shoots as compared to uninoculated control. In general, biofilm of isolate A 13 resulted in higher accumulation of malic acid, succinic acid and propionic acid. However, for isolate CR 24, inoculation of biofilmed formulation led to decrease accumulation of acids as compared to only *Rhizobium* inoculation. Interesting results were obtained for accumulation of formic acid. At both the doses of pathogen, the control treatment showed negligible accumulation of formic acid. However at lower dose of pathogen (15g pot^{-1}), inoculation of *Rhizobium* or its biofilm showed significantly higher concentration of formic acid as compared to its accumulation at higher dose of pathogen (30 g pot^{-1}). At higher dose of pathogen, formic acid was accumulated only in treatments inoculated with *Rhizobium* A 13 and its biofilm indicating its role in induction of resistance to pathogen. Peroxidase enzyme activity also showed variations among the treatments. For both isolates, the biofilm formulations showed higher activity of peroxidase as compared to single inoculation of *Rhizobium*. CR 24 and its biofilm recorded higher activity as compared to A 13 and its biofilm. Role of defense enzymes is well known as protection mechanism against pathogens. Rhizobia have been reported to induce systemic resistance in host plants and provide protection against various phytopathogens. Increased activity of peroxidase in inoculated plants as compared to uninoculated plants may be the result of induced systemic resistance by rhizobial isolates.

1.7. Proteomic analysis of diversified Rhizobium isolates to identify functionally important proteins (BAU)

A total of 116 strains of rhizobia have been characterized from acid soils of Jharkhand nodulating pigeonpea, groundnut, black gram, soybean, cowpea, mung, pea, chickpea, french bean, broad bean, berseem and lentil respectively. Using 16S rRNA analysis, novel strains of *Rhizobium* of pigeon pea tolerant to acidic soil pH regimes were identified (Table 6).

Table 6. Pigeonpea isolates from acidic soils of Jharkhand

Isolate ID	District	Soil pH	GenBank Acc. No.
Rhizobium sp.2(2) BAU	Jamtara	5.5	KF309195
Rhizobium sp.35BAU	Deoghar	4.5	KF309203
Rhizobium sp.351BAU	Deoghar	4.5	KF309204

Detailed analysis at proteomic level of various *Rhizobium* isolates was done by "Two-Dimensional Gel Electrophoresis (2-DE)" followed by MALDI-TOF-TOF (Peptide-Mass Fingerprinting) to characterize several important "unique" protein differences amongst acid-tolerant *Rhizobium* isolates from pigeonpea. Analysis of 14 (Fourteen) "unique" protein spots identified the genes implicated in the acid-soil tolerance (Appendix 1). Analysis of 10 (Ten) "unique" protein differences characterized from acid-tolerant *vs* samples from Vertisols for *Rhizobium* isolates in soybean (Fig 8) and chickpea revealed identity of the genes implicated in wide dimension of varied functions (Appendix 1). MALDI-TOF-MS is an important tool for protein identification, because of its high throughput, sensitivity, and high mass accuracy. The analysis of "Candidate Proteins" by "Mass Spectrophotometry (MS)" followed by "Peptide Mass Fingerprinting" analysis revealed corresponding genes to be involved in various pathways of the cellular metabolism. The analysis has elucidated identities of the corresponding genes with respect to the "Signature Proteins" implicated in the adaptation of the isolates to various acidic soil pH regimes. The "Proteome Maps" generated in the analysis pin-point to the genes which may be contributing towards imparting selective acid soil tolerance amongst various *Rhizobium* isolates analysed.

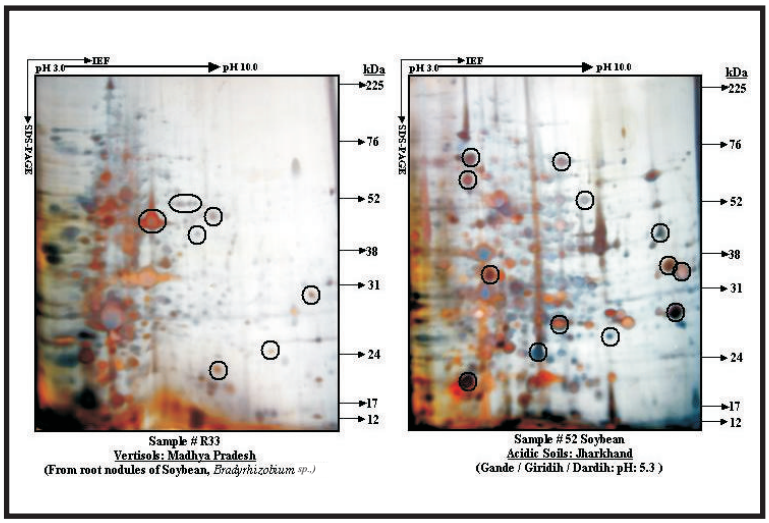


Fig 8. 2-D gel electrophoresis profiles of *Rhizobium* isolates of soybean from Vertisols of Madhya Pradesh and Jharkhand. The circled protein spots depict, "*unique spots*", not present in either of the corresponding samples analysed.

1.8. Molecular characterization of Potassium Solubilizing Bacteria (AAU)

Twelve bacterial isolates were screened from the rhizospheres of banana, rice and hot chilli based on clear zone formation on Aleksandrov agar medium (mica @ 0.5 %). The *in vitro* solubilization by the isolates resulted in significant release of K (9.1-27.1 µg mL^{-1}) at 45 d of incubation due to decrease in pH (initial 7.0 to 4.6-5.3). Five isolates $KSBB_3$, $KSBB_8$, $KSBB_9$, $KSBC_5$ and $KSBC_6$

obtained from banana and chilli rhizospheres were subjected to quantitative estimation of NH_4OAc-K release from soil. The results illustrated the release of NH_4OAc-K, in between 17.1-42.0 mg kg^{-1} after 50 d of incubation in soil by the selected isolates. Identification of the isolates was done by 16S rRNA (1500bp) gene sequencing as *Bacillus cereus* (KSBB3, $KSBB_8$) and *Klebsiella spp.* ($KSBB_9$, $KSBC_5$ and $KSBC_6$) (Table 7)

Table 7. Identification of the selected isolates by 16S rRNA gene sequencing

Isolates	Identification(BLAST)	Similarity (%)
$KSBB_3$	*Bacillus cereus* strain NAPCC-1	99
$KSBB_8$	*Bacillus cereus* strain BRL-02-31	99
$KSBB_9$	*Klebsiella variicola* strain kms0422	98
$KSBC_5$	*Klebsiella* sp 2009110	98
$KSBC_6$	*Klebsiella* sp	96

1.9. Evaluation of Fluorescent Pseudomonads for Disease Suppression in Groundnut (DGR)

Effect on growth and yield of groundnut

DAPG-producing fluorescent pseudomonads having multiple plant growth promoting traits (production of IAA and siderophore; P-solubilization, ammonification and ACC deaminase activity) besides suppressing soil-borne fungal pathogens like *A. niger*, *A. flavus* and *S. rolfsii* were selected after initial screening in pots for field trial for evaluating their effect on the growth and yield of groundnut cv. TG37A with recommended doses of fertilizers. Inoculation of DAPG-producing fluorescent pseudomonads improved plant growth and biomass in most of the cases over uninoculated control at harvest. Application of *P. fluorescens* FP98 significantly improved the pod and haulm yield over uninoculated control across seasons of summer 2014 & 2015 and kharif 2014 (Table 8). In case of haulm yield, significant improvement was achieved with the application of *P. putida* DAPG1 and *P. fluorescens* FP98 over seasons and over uninoculated control (Table 8). Inoculation resulted in enhanced pod yield by 13-17% during summer 2015 and 15% during summer 2014. During kharif 2014 inoculation enhanced pod yield by 10%. These DAPG-producing fluorescent pseudomonads are being evaluated in AICRP-G centres.

Table 8. Evaluation of the effect of application of DAPG-producing fluorescent pseudomonads on the growth and yield of groundnut

Trt.	Pod yield (kg ha^{-1})			Haulm yield (kg ha^{-1})			Shelling out-turn (%)		
	Kharif	Summer	Summer	Kharif	Summer	Summer	Kharif	Summer	Summer
	2014	2014	2015	2014	2014	2015	2014	2014	2015
Control	2957	1915	1824	3699	5240	4107	67.8	59.3	64.2
DAPG1	3237	1929	2059	4088	5965	4621	70.4	59.8	68.2
DAPG2	3039	1879	1939	4063	5068	4415	67.6	63.4	63.9
DAPG3	2829	1872	1690	3974	6510	4179	68.4	60.4	62.5
DAPG4	3275	1784	2137	4228	5678	4505	70.5	64.2	67.7
DAPG5	3030	1918	1768	3695	5040	4076	68.7	57.3	61.6
DAPG6	3026	2213	2110	4207	6948	4361	66.5	62.3	67.3
FP98	3283	2198	2109	4102	6618	4406	69.9	61.8	68.2
LSD (p=0.05)	187	186	200	299	513	246	2.4	2.4	2.2

Development of suppressive soils

To make soils naturally suppressive to soil-borne fungal pathogens like *Sclerotium rolfsii* causing stem rot in groundnut, DAPG-producing fluorescent pseudomonads, highly antagonistic to *S. rolfsii*, were applied and evaluated with the susceptible cultivar GG20 during kharif 2014 and 2015 (Table 9). The application of the DAPG-producing fluorescent pseudomonads suppressed the seedling mortality of groundnut, cultivar GG20 from 70-80% in pathogen control to 20-40% in treatments inoculated with different DAPG-producing fluorescent pseudomonads. *P. fluorescens* FP82 was the best in both the seasons in suppressing the incidence of the stem rot of groundnut caused by *S. rolfsii*.

Table 9. Effect of application of DAPG-producing fluorescent pseudomonads on the mortality of groundnut seedlings due to infection of *Sclerotium rolfsii*

Treatments	% Seedling mortality	
	Kharif 2014	Kharif 2015
Control	6.7	9.3
Pathogen (P)	82.2	73.2
P+DAPG2	28.9	29.3
P+DAPG3	30.1	28.9
P+DAPG4	24.5	24.3
P+DAPG7	31.3	38.1
P+FP20	26.8	36.8
P+FP46	28.3	37.2
P+FP86	29.2	24.5
P+FP93	30.2	28.7
P+FP94	25.7	22.8
P+FP121	28.6	29.6
P+FP133	27.4	32.5
P+FP82	23.1	21.3

The yield data (normalized data) showed pod yield of groundnut, cultivar GG20 was enhanced by 10 to 16% during kharif 2014 (Table 10) and 13-22% during kharif 2015. These DAPG-producing fluorescent pseudomonads have also been evaluated at different AICRP (G) centres throughout the country.

DAPG-producing fluorescent pseudomonads, identified under AINP-Biofertilizer programme, were evaluated through AICRP(G) centres during kharif 2014 at Shirgaon, Junagadh (JAU), Jagtial, Jalgaon, Chintamani, Tirupati, Bhubaneshwar and Mohanpur. Not considering trials with low or very low control yields, increase in pod yield ranged from 20-40% averaging 30%. Besides, the incidence of collar and stem rot was also reduced appreciably.

Table 10. Enhancement in yield of groundnut GG-20 by DAPG-producing fluorescent pseudomonads

Treatments	Haulm yield		Pod yield (kg/ha)		Shelling out-turn (%)		Hundred Kernel Mass (g)	
	Kharif 2014	Kharif 2015	Kharif 2014	Kharif 2015	Kharif 2014	Kharif 2015	Kharif 2014	Kharif 2015
Control	3855	5499	2512	2397	63.7	66.00	41.36	48.77
Pathogen (P)	3753	5480	2574	2191	64.8	66.80	42.17	49.07
P+DAPG2	4145	6099	2650	2307	63.6	65.20	41.97	48.77
P+DAPG3	3964	6096	2428	2343	64.8	68.03	43.39	50.83
P+DAPG4	4111	6236	2405	2739	66.4	67.60	43.56	49.33
P+DAPG7	4208	5654	2579	2921	66.6	69.60	43.39	51.60
P+FP20	4286	5344	2635	2737	67.5	67.67	43.49	52.83
P+FP46	4448	5401	2861	2564	66.1	66.70	44.61	49.57
P+FP86	4352	6386	2817	2622	67.2	68.10	44.92	52.57
P+FP93	4136	5780	2899	2594	62.2	67.93	42.45	49.33
P+FP94	4142	6497	2710	2756	64.4	66.10	40.97	49.50
P+FP121	3866	6265	2876	2703	65.9	66.97	42.33	51.43
P+FP133	3993	5874	2909	2750	66.6	67.37	39.13	49.93
P+FP82	4201	5776	2755	2604	65.2	68.63	43.49	50.83
LSD (p=0.05)	331	427	239	294	2.64	2.13	2.15	NS

1.10. Development of multifunctional microbial inoculum for upland rice based cropping system (CRURRS)

Improving nucleus inoculum of native AM fungal consortium

Work in previous few years showed that mass inoculum (MI) of AM fungal consortium produced by multiplying nucleus inoculum (NI) developed on substrate mixture of vermiculite: soil: FYM in the ratio of 75:25:5 fortified with Hoagland solution @ 10 ml/100 g/week (for 4 weeks) substantially improved the efficacy of MI in terms of reducing the effective dose of application by half (from 1 t ha^{-1} to 0.5 t ha^{-1}). Further attempts were made to improve efficacy of MI by increasing AMF spore

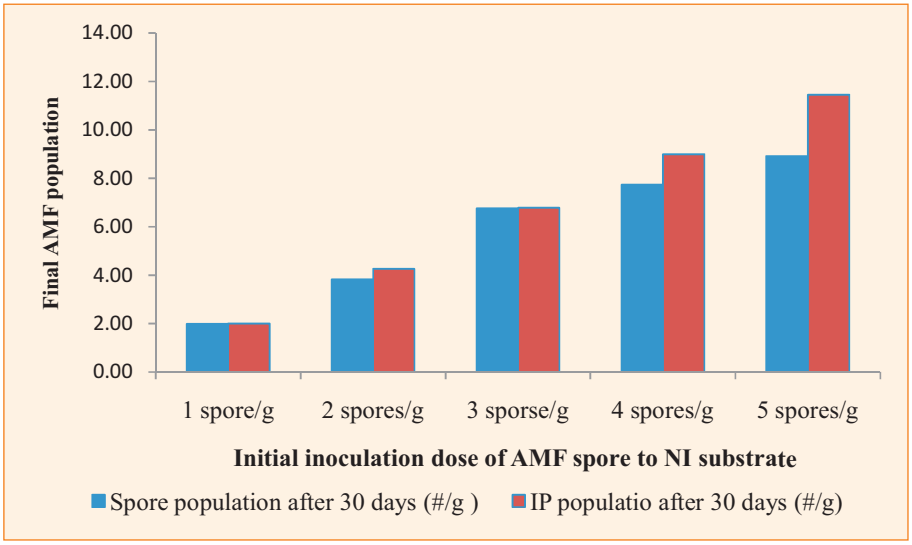


Fig 9. Progressive increase in final population (AMF) with increased nucleus inoculum dose.

density in NI from 1 spore/g substrate to 2, 3, 4 & 5 spores g^{-1}. After 30 days of inoculation of sorghum plants, a progressive and proportionate increase was seen in spore and infective propagule population with increase in initial spore inoculation dose (Fig 9). These improved inocula will be tested in field.

Characterization of non-symbiotic fungal endophytes

Ten non-symbiotic fungal endophytes were isolated following standard protocol, from rice roots collected from various upland sites of Jharkhand. Out of the 10, six were screened in glass house for their ability to impart moisture stress tolerance to direct seeded upland rice (cv. Kalinga III). Based on morphological features the three endophytes were identified as *Aspergillus* sp. (E-1), *Trichoderma* sp. (E-2) and *Fusarium* sp. (E-3). Soil moisture in the experimental pots was maintained at 25% of WHC of the soil until 35 days after germination (DAE) after which moisture stress was imposed by withdrawing watering (in treatments with stress). Dry matter production was measured at 45 DAE. Treatments without endophyte inoculation and without moisture stress were maintained in each set. Under moisture stress, average soil moisture at 45 DAE was 12.2% as compared to that of 18.1% in no-stress treatments. While all the stress (moisture) treatments led to reduction in dry matter production, the extent of reduction were less when inoculated with E-1, E-2, E-3 and E-6 over control (no-inoculation) with least reduction in E-3 (*Fusarium* sp.) followed by E-1 (*Aspergillus* sp.) (Fig 10).

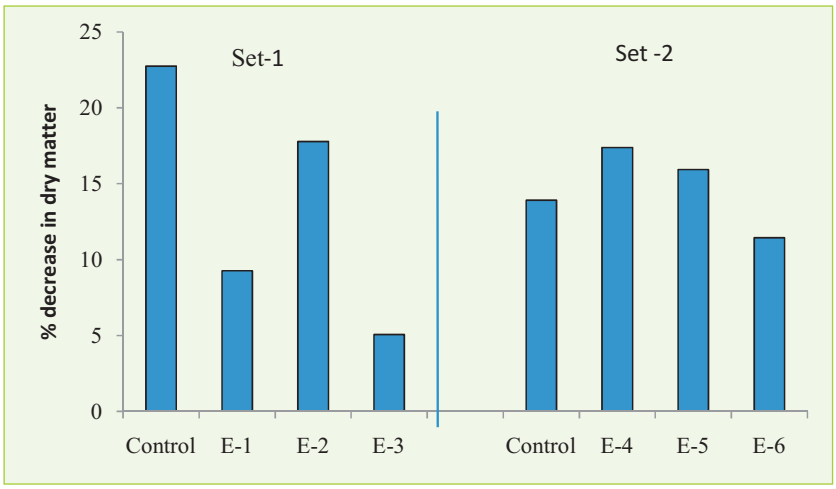


Fig 10. Differential effects of moisture stress on rice (cv. Kalinga III) at vegetative state (35-45 DAE) inoculated with some fungal entophytes (E-1 to E-6).

1.11. Development of Actinomycetes Formulations and Testing on Field Crops (IISS, JNKVV)

Liquid based formulation of 17 isolates of actinomycetes of IISS, Bhopal were tested on soybean in kharif 2014 and wheat during rabi 2014-15 and consortia of best performing actinomycetes isolates with *Rhizobium* and PGPR on chickpea during rabi 2014-15. Two controls *viz.,* fertilized uninoculated control (FUI) and unfertilized uninoculated control (UFUI), were included for comparison.

Soybean

In Kharif 2014, of the 17 isolates, 13 were able to increase the grain yield significantly over FUI. Isolate A10 was most efficient in improving nodulation and yield. Average increase in grain and straw yield was 41% and 56% respectively over FUI. Uptake of N, P and K by the crop was enhanced by 86.8, 5.4 and 50.9 kg ha^{-1} (Table 11).

Table 11. Evaluation of actinomycetes isolates on soybean yield and NPK uptake in Vertisol

	Nodulation $plant^{-1}$		Yield (kg ha^{-1})		Total nutrient uptake by crop (kg ha^{-1})		
	No.	Dry wt.(g)	Grain	Straw	N	P	K
17 isolates range	26-45	0.19-0.27	1444-2528	2781-4983	151-277	8.8-15.9	88-161
Mean	37	0.22	2003	3951	203	12.5	125
FUI	30	0.16	1417	2521	116	7.1	74
UFUI	25	0.14	1194	2332	97	5.7	63
LSD (p=0.05)	13.68	0.10	413.40	1311.88	58.02	2.54	34.36
CV (%)	22.8	27.8	13.0	21.0	18.3	13.0	17.5

Wheat

In rabi 2014-15 on wheat (Fig 11), isolate A10 and A4 isolates increased the grain and straw yields significantly over FUI. Average increase in grain and straw yield by all the isolates was 22% and 17% higher over FUI (Table 12). Uptake of N, P and K by the crop was enhanced by 50, 14 and 50 kg ha^{-1}. Isolate A10 was found best among all isolates in increasing NPK uptake.

Table 12. Evaluation of actinomycetes isolates on wheat yield and NPK uptake in Vertisol

	Yield (kg ha^{-1})		Total nutrient uptake by crop (kg ha^{-1})		
	Grain	Straw	N	P	K
Mean of 17 isolates	4827	6856	119	49	109
Range	4067-5095	5984-7012	96-143	41-59	94-127
FUI	3961	5878	69	35	77
UFUI	2822	4739	47	25	59
LSD (p=0.05)	916	916	34.40	9.66	19.21
CV%	12.4	8.7	19.2	13.0	11.5

Chickpea

In rabi 2014-15 (Fig 11), the best consortium of Rhizobia and PGPR (*Rhizobium* and PGPR) +Actinomycetes A (A10) + Actinomycetes B (A17) along with recommended dose of NPK gave maximum response over FUI in terms of nodulation; grain and straw yield which increased by 77% and 125% respectively (Table 13).

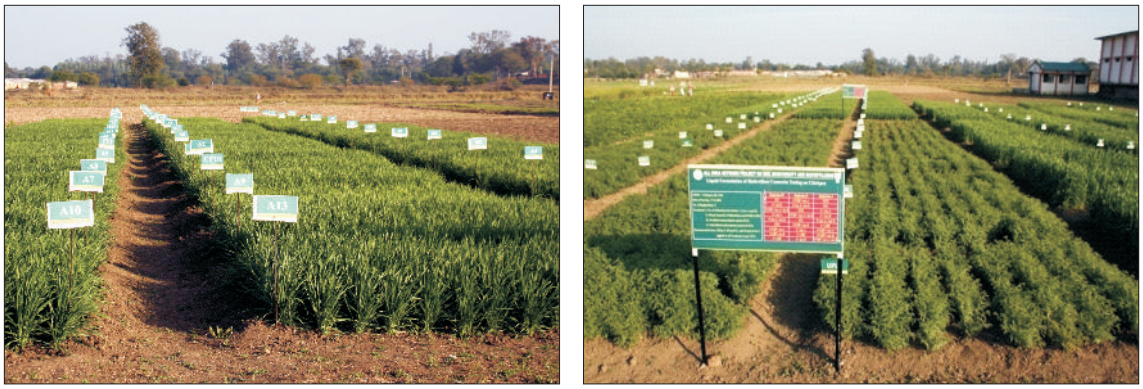


Fig 11. Evaluation of Liquid Formulation based Consortia on wheat and chickpea in field.

Table 13. Effect of liquid formulation based consortia on Chickpea yield and NPK uptake in Vertisol

Treatment	Nodulation plant^{-1}		Yield (kg ha^{-1})		Total nutrient uptake by crop (kg ha^{-1})		
	No.	DW (mg)	Grain	Straw	N	P	K
F+Act. A	15	37	861	1141	35	5.3	13.9
F+Act. B	13	35	833	1247	36	5.6	14.3
F+Act. A + Act. B	17	34	1011	1671	49	7.6	18.5
F+CRP	14	30	911	1475	41	6.8	17.2
F+CRP + Act. A	14	51	1261	2098	64	10.2	24.3
F+CRP + Act. B	15	55	1317	2374	71	11.0	27.0
F+CRP + Act. A+Act. B	18	55	1400	2477	79	12.6	28.8
FUI	13	29	789	1103	32	4.6	12.6
UFUI	12	26	661	1068	27	3.9	10.6
LSD (p=0.05)	6.0	2	422	218	17	3.2	4.6
CV (%)	25.1	33.2	25.5	8.1	21.1	25.5	14.9

Table 14. Effect of liquid formulation based consortia on soybean yield and NPK uptake in Vertisol

Treatment	Nodulation plant^{-1}		Yield (kg ha^{-1})		Total nutrient uptake (kg ha^{-1})		
	No.	Dry wt.(g)	Grain	Straw	N	P	K
F+Act.A	20	0.46	984	1531	78	5.4	49
F+Act.B	22	0.46	989	1583	79	5.5	52
F+Act.A+F+Act.B	22	0.54	995	1632	83	5.9	56
F+CRP	27	0.57	1000	1648	86	6.1	57
F+CRP+Act.A	29	0.62	1039	1838	92	7.1	65
F+CRP+Act.B	30	0.64	1122	1968	101	7.8	69
F+CRP+Act.A+Act.B	32	0.71	1194	2207	111	9.0	81
FUI	18	0.46	972	1342	73	4.8	44
UFUI	16	0.39	716	1246	53	3.4	36
LSD (p=0.05)	4	0.22	431	491	26	1.9	15
CV (%)	10.1	24.7	26.1	17.9	18.5	18.7	16.1

Soybean

In kharif 2015, the best consortium of Rhizobia and PGPR (*Rhizobium* and PGPR) +Actinomycetes A (A10) + Actinomycetes B (A17) along with recommended dose of NPK gave maximum response over FUI in terms of nodulation (nodule mass increased by 54%) and grain yield (+23%) and NPK uptake (Table 14).

1.12. Diversity of Arthrobacter in Vertisols and testing on field crops (IISS, JNKVV)

Arthrobacter spp. are major representatives of the cultural fraction of soil bacteria. To explore them for providing better ecosystem services as well as for use as inoculants in agriculture they were isolated from various soils (Fig 12) under different cropping systems at peak vegetative stage in kharif and rabi season. *Arthrobacter* were enumerated on *Arthrobacter* medium with addition of methyl red (150 µg ml^{-1}), NaCl (2%) and cycloheximide (0.01%). Data in Table 15 shows the numbers of various microbial groups in rabi season.

Table 15. Microbial abundance (CFU ± SEm) in the rhizospheric samples of different sampling sites

Site	Bacteria ($\times 10^6$)	Fungi ($\times 10^3$)	Actinomycetes ($\times 10^6$)	*Arthrobacter* ($\times 10^5$)
Geelakhedi	28.0 ± 4.4	5.7 ± 2.1	17.1 ± 0.5	0.77 ± 0.2
Jabalpur	43.7 ± 3.8	16.3 ± 4.9	21.2 ± 7.2	22.0 ± 2.8
Chhindwara	20.3 ± 3.1	9.7 ± 1.5	17.8 ± 2.3	1.2 ± 0.5
Bhopal	27.3 ± 3.8	5 ± 3.5	25.0 ± 3.7	29.7 ± 2.3

Based on colony morphology, growth rate, pigmentation, polysaccharide production etc., diversity of *Arthrobacter* isolates (Fig 12) followed the trend of Chickpea (Jabalpur) $\geq$ Chickpea (Bhopal) > maize (Chhindwara) > soybean (Geelakhedi) $\geq$ soybean (Bhopal) > Wheat (Chhindwara) = Wheat (Geelakhedi) > Rice (Jabalpur) (50, 50, 30, 20, 20, 12, 12 and 5 morpho-types respectively). A total of 200 isolates were screened for growth promotion of maize seedlings and PGPR characteristics like indole acetic acid production, siderophore production and phosphate solubilization. The characteristics of 13 effective strains and one ineffective strain are listed in Table 16 below. These fourteen strains have been shortlisted for sequencing of 16S rRNA gene.

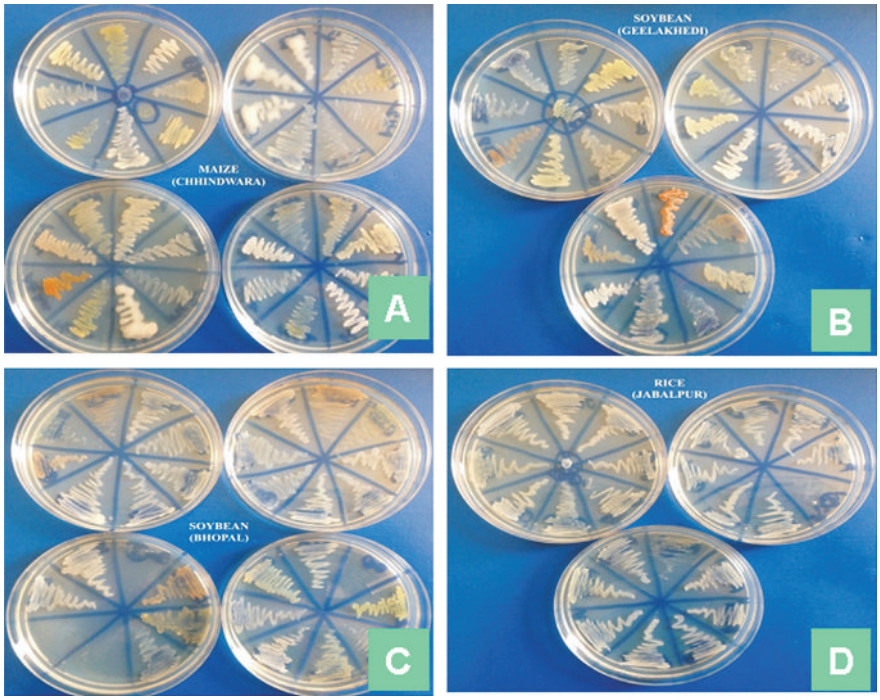


Fig 12. Cultural diversity of *Arthrobacter* spp. in different cropping system. A- Maize (Chhindwara), B- Soybean (Geelakhedi), C- Soybean (Bhopal), D- Rice (Jabalpur).

Table 16. PGPR attributes of 14 *Arthrobacter* strains

Rhizosphere		Place	pH	Organic Carbon (%)	PGPR Attributes				
					PO_4 solubilization	Siderophore production	IAA (µg/ml)	Vigor index	% Increase of vigor index
AR1	Chickpea	Bhopal	7.5	0.59	++	++	10.1	4700	51.6
AR2	Soybean	Geelakhedi, Rajgarh	7.5	0.81	-	+++	11.8	4730	52.3
AR3	Chickpea	Jabalpur	5.6	0.94	+	+	0.4	5230	68.7
AR4	Soybean	Geelakhedi, Rajgarh	7.5	0.81	-	-	10.6	5940	91.6
AR5	Soybean	Bhopal	7.4	0.59	-	+++	1.7	5490	77.1
AR6	Soybean	Bhopal	7.4	0.59	-	-	7.8	5740	85.2
AR7	Wheat	Chhindwara	7.1	0.86	-	+	4.7	5540	78.7
AR8	Wheat	Geelakhedi, Rajgarh	8.0	0.85	-	++	3.7	5440	75.5
AR9	Maize	Chhindwara	7.7	0.76	-	-	5.9	5481	76.8
AR10	Rice	Jabalpur	6.6	1.21	-	-	2.0	4650	50.0
AR11	Chickpea	Bhopal	7.5	0.59	-	-	0.6	4710	51.9
AR12	Soybean	Bhopal	7.4	0.59	-	NA	NA	1140	-63.2
AR13	Soybean	Geelakhedi, Rajgarh	7.5	0.81	-	-	1.6	4740	52.9
AR14	Soybean	Bhopal	7.4	0.59	-	-	6.5	5880	89.7

Evaluation of Liquid formulations of *Arthrobacter*

Thirteen isolates of *Arthrobacter* liquid based formulations of IISS, Bhopal were tested on rice, maize and soybean in Vertisols during kharif 2015 at JNKVV, Jabalpur. Two controls *viz.,* fertilized uninoculated control (FUI) and unfertilized uninoculated controls (UFUI) were also included.

All the isolates of *Arthrobacter* increased the grain yield of rice over FUI (Table 17); the isolates AR6, AR8 and AR10 gave 28, 27 and 26% higher grain yield. All isolates improved N, P and K uptake. Five isolates - AR6, AR8, AR10, AR5 and AR1 gave higher nutrient uptake.

Table 17. Effect of *Arthrobacter* inoculation on yield and nutrient uptake by rice in Vertisol

	Yield (kg ha^{-1})		Total uptake of nutrient by crop (kg ha^{-1})		
	Grain	Straw	N	P	K
Range of 13 isolates	4067-5151	4704-6284	96-140	24-38	60-89
Mean	4650	5582	119	32	75
FUI	4000	4805	93	23	59
UFUI	3833	4582	82	22	50
LSD (p=0.05)	971	1434	28	9	22
CV%	12.9	15.9	15.1	17.2	18.5

All *Arthrobacter* isolates increased the grain yield of maize (Table 18) but only AR3, AR4, AR6, AR7, AR10, AR11 and AR12 were found significantly better giving about 32% yield increase. Most isolates increased total uptake of N, P and K. Most significant results were obtained with AR10, AR3 and AR12 which gave increased NPK uptake averaging 40% over the respective FUI.

Table 18. Effect of *Arthrobacter* inoculation on yield and nutrient uptake by maize in Vertisol

Isolate No.	Yield (kg ha^{-1})		Total uptake of nutrient by crop (kg ha^{-1})		
	Grain	Stover	N	P	K
Range of 13 isolates	2116-3043	7923-10991	108-156	30-45	81-121
Mean	2500	9712	132	38	102
FUI	2108	8943	111	31	88
UFUI	1211	4722	58	15	46
LSD (p=0.05)	229	1622	20	4	16
CV%	5.8	10.5	9.4	7.6	10.2

In soybean, most of the strains could not increase nodule number or nodule mass significantly. Three isolates of AR2, AR4 and AR7 gave significant increase soybean grain yield by 27, 26 and 24%, respectively over FUI (1301 kg ha^{-1}). All isolates of *Arthrobacter* increased total NPK uptake (Table 19), of these AR2, AR4 and AR7 significantly increased nutrient uptake by the crop, N uptake by 35-70%, P uptake by 18% and K uptake by 40-45% over FUI.

Table 19. Effect of *Arthrobacter* inoculation on nodulation, yield and nutrient uptake by soybean in Vertisol

	Nodulation plant^{-1}		Yield (kg ha^{-1})		Total uptake of nutrient by crop (kg ha^{-1})		
	No.	Dry wt.(g)	Grain	Straw	N	P	K
Range of 13 isolates	19-28	0-17-0.22	1301-1658	5234-6842	193-286	12-19	81-142
Mean	22	0.20	1461	5967	220	14	109
FUI	21	0.17	1301	5211	165	11	87
UFUI	17	0.14	1012	3787	107	7	47
LSD (p=0.05)	6	0.06	236	1694	46	4	29
CV%	17.1	18.8	10	17.7	13.3	17.3	17.2

1.13. Development of biofertilizer technologies for temperate fruits, vegetables and medicinal plants (YSPUHF)

Sweet Cherry

An improved method of raising nursery plantlets in nethouse consisting of conjoint application of PGPR (*Bacillus* spp.) and AMF consortia (AMF-II+P7) at 75 % NP showed a significant increase in shoot and root length and total biomass production over conventional method (Table 20 and Fig 13).

Table 20. Effect of co-inoculation of PGPR and AMF consortia on growth of sweet cherry at six months

Treatments	Shoot Length (cm)	Root Length (cm)	Plant biomass (g/plant)
Uninoculated	44.8	19.7	23.6
100 % RDF	52.6	25.3	33.6
75% NP + *Bacillus* spp.	60.6	32.7	48.7
75% P + AMF II	58.5	35.8	46.7
75% NP + *Bacillus* spp. + AMF II	83.4	39.1	62.5
LSD (p = 0.05)	4.2	2.7	0.7

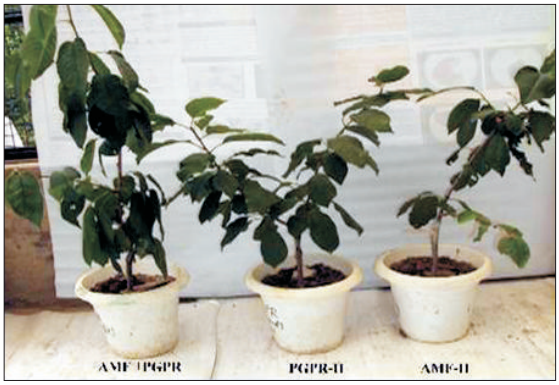


Fig 13. Effect of co-inoculation (PGPR and AMF consortia) and chemical fertilizers (75% RDF) on growth of sweet cherry

Tomato

Six indigenous isolates of *Bacillus* spp. with multipurpose PGP traits were screened under field conditions. The isolate *B. subtilis* T1 gave highest increase in plant biomass (100%), fruit number (55%) and fruit yield (70 %) over uninoculated control (Fig 14).

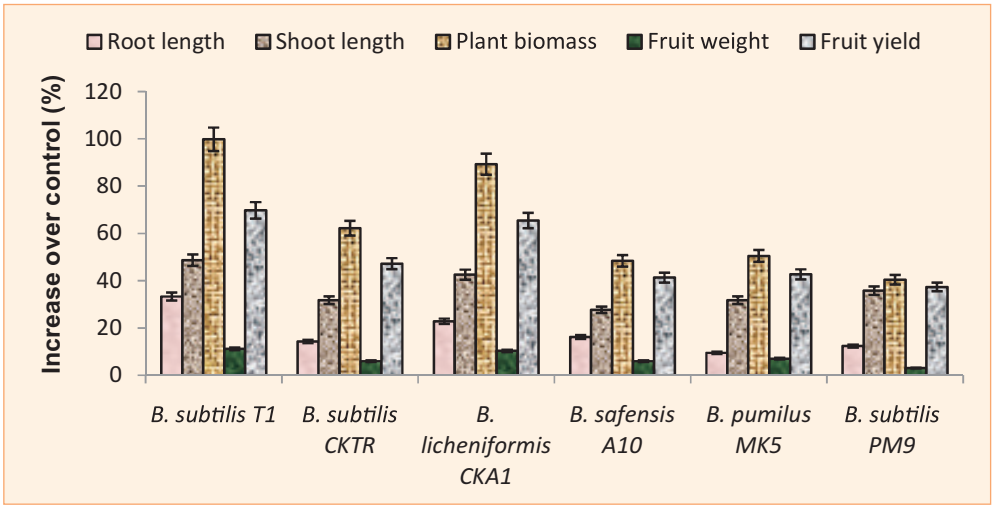


Fig 14. Increase in plant growth parameters and tomato yield over uninoculated control.

Capsicum

The application of indigenous PGPR and chemical fertilizers gave significant increases in plant biomass and fruit yield. At 80% RDF application, *Bacillus amyloliquefaciens* and *Pseudomonas aeruginosa* gave 18-19 Mg fruit yield ha^{-1}. Overall the isolates gave about 30% increase in fruit yield over 100% RDF besides saving of 20% N and P chemical fertilizers (Table 21).

Table 21. Effect of inoculating PGPR on the growth and yield of capsicum in field

Treatment	Plant height (cm)	Plant biomass (kg ha^{-1})	Number of fruits (plant^{-1})	Yield/plant (t ha^{-1})
100% RDF	66.0	2864.8	8.4	12.6
80% RDF	58.3	2411.6	6.6	9.5
80% RDF + *Pseudomonas aeruginosa*	68.4	3266.4	9.5	18.1
80% RDF + *Bacillus amyloliquefaciens*	70.2	3427.7	9.8	19.1
80% RDF + *Bacillus* spp.	62.5	3067.7	8.7	15.3
80% RDF + *Bacillus* spp.	63.4	3028.7	8.5	14.8
80% RD + *Bacillus* spp. - Ref. strain	65.8	3036.4	8.3	14.2
LSD (p = 0.05)	3.8	264.3	0.6	2.1

Seabuckthorn (*Hippophae rhamnoides*)

A total of 106 P-solubilizing isolates were obtained from two locations from Lahaul & Spiti district of Himachal Pradesh of which fourteen bacterial isolates were selected for further testing based on P-solubilization, siderophore production and IAA production.

Apple

The effect of *Bacillus licheniformis* inoculation (by drenching of apple plant basin with one litre of liquid formulation diluted to five liters) was demonstrated in the fields at three different locations of Shimla district (Fig 15). Increased yields of apple ranging from 30 to 50% were obtained at all the five locations over uninoculated control (Table 22).

Fig 15. An overview of Apple orchards inoculated with *Bacillus licheniformis.*

Table 22. Effect of inoculating *Bacillus licheniformis* on growth and yield of apple at different locations (2015-16) in farmers' fields

Treatments	Apple yield (kg tree^{-1})		
	Matiana (Sabloab)	Thanedar (Shatla)	Kotkhai (Kyari)
Uninoculated Control	85	60	59
Bacillus licheniformis	112 (33)	90 (50)	84 (43)

*Figures in parenthesis are percent increase over control

Isolation of *Frankia* spp.

Root nodules of *Casuarina equisetifolia* and *Alnus nitida* collected from Solan (Kunihar, Nalagarh and Nauni) and Kullu (Patlikuhl, Manali and Bajaura) in H.P. were used for isolation of *Frankia* spp. on different medium viz., DPM- Defined Propionate Minimal Medium, Qmod Agar medium, BAP medium and solid P medium. After incubation for 3-4 weeks, 24 isolates showing typical starfish shape colonies and no turbidity in liquid medium (Fig 16) were purified and screened for growth on N-free medium. All the isolates were positive for P-solubilization and nitrogen fixation but only five isolates showed HCN production (Fig 17).

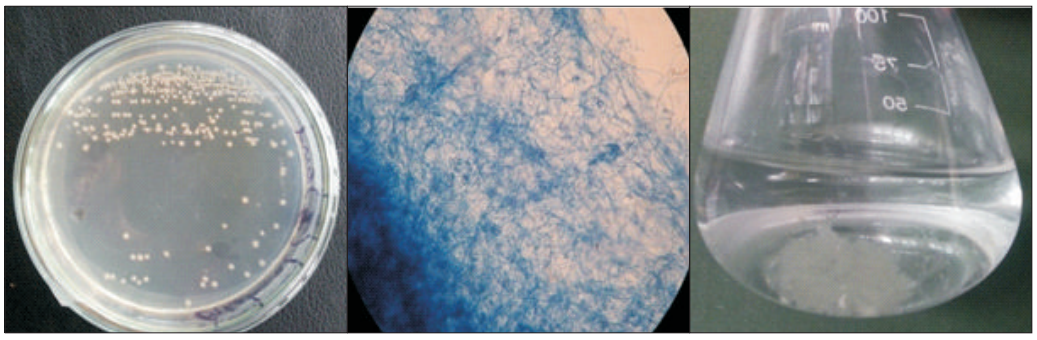


Fig 16. Culture plate of *Frankia* on DPM medium (Left); Morphology of *Frankia* after staining with trypan blue in lactophenol (Middle); Growth in liquid medium (Right).

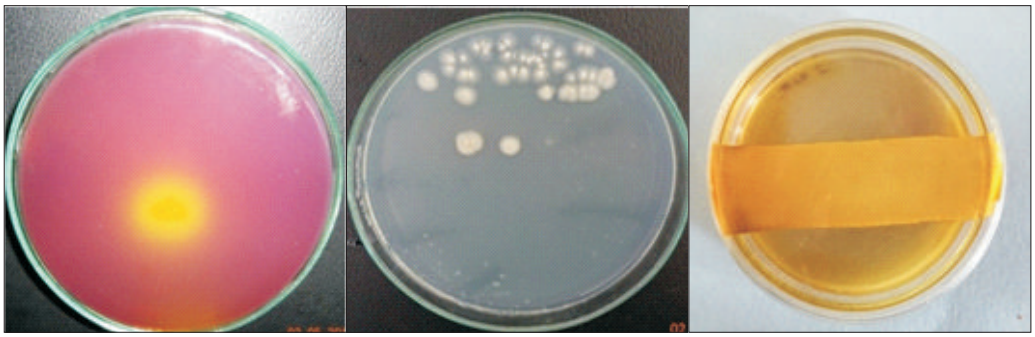


Fig 17. Screening of *Frankia* for Plant growth promoting traits: P-solubilization; Nitrogen fixation and HCN production.

1.14. Biofertilizers for Tropical Vegetables in Acid Soils (OUAT)

Long term Biofertilization Experiment

In a long term experiment on using biofertilizers in acid alfisols started during *kharif* 2010, green gram, maize, cabbage, cowpea and ragi were grown in sequence (11th to 15th crop) in fixed plots with 10 treatments of fertilizers applied (Table 23) as per soil test dose (STD), FYM, Vermicompost, Lime, and Biofertilizers. The residues of each crop (after harvest of economic produce) were incorporated *in situ*. The source of organics was FYM or VC, applied @ 5 and 2.5 t ha^{-1} respectively. The biofertilizers for pulses (or pulse- vegetable) was *Rhizobium* applied as seed inoculation and for cereals and vegetables were diazotrophs (*Azotobacter, Azospirillum* and phosphorus solubilizing microorganisms (PSM) applied in 1:1:1 ratio @ 4 kg each ha^{-1} inoculated to pre-limed (5 %) vermicompost or FYM in 1:25 ratio, incubated for 7 days at 30 per cent moisture, applied on the day of sowing or planting of crops. Lime was applied as paper mill sludge @ 0.1 LR for maize and ragi and @ 0.2 LR for green gram, cowpea and cabbage at the time of sowing of seeds in the rhizosphere and one day before planting of cabbage seedlings.

Table 23. Nutrients added (kg ha^{-1}) to five different crops grown in sequence

	Inorganic				Organic (FYM/VC)				Total			
Crop	**N**	**P**	**K**	**S**	**N**	**P**	**K**	**S**	**N**	**P**	**K**	**S**
Green gram	20	13	30	20	20	10	15	7	40	23	45	27
Maize	150	22	60	33	25	12	20	7	175	34	80	40
Cabbage	156	18	40	20	25	12	20	7	185	30	60	27
Cowpea	40	18	40	20	25	12	20	7	65	30	60	27
Ragi	52	15	32	10	15	7.5	11	4.5	67	22.5	43	14.5

There was less production of economic produce with fertilizers alone as compared to STD + Organics which followed the order: ragi (10 %) < maize (13 %) < green gram (33 %) < cabbage (55 %) < cowpea (59 %). Integrating BFs application with STD + organics increased the economic produces ranging from 10 to 14 per cent. Ameliorating acid soils with lime application further increased the produce/yield ranging from 20-27 % (Table 24).

Table 24. Productivity of crops (Mg ha^{-1}) under the influence of long term INM with Biofertilizers

INM practices	Green gram	Maize	Cabbage	Cowpea	Ragi
Absolute control	0.32	1.60	1.30	0.11	1.14
BFs	0.42	2.90	1.70	0.12	1.37
Half STD + BF	0.54	5.83	5.40	0.18	1.79
STD	0.54(-33)	5.47(-13)	18.60(-57)	0.26(-59)	2.16(-10)
STD + organics	0.80	6.30	43.5	0.63	2.40
STD +Organics + BF	0.89(12)	7.00(11)	48.1(11)	0.72(14)	2.64(10)
STD +Organics + L + BF	0.96(20)	7.60(20)	52.6(21)	0.80(27)	2.88(20)
LSD (p=0.05)	0.037	0.30	1.8	0.03	0.12

*Data in the parenthesis indicate per cent decrease or increase compared over STD + organics

The crops generated biomass ranging from 2 Mg ha^{-1} to 47 Mg ha^{-1} (Table 25). Omitting organics addition with STD as package of practice resulted in loss of biomass in all crops except for ragi. However, integration of BFs with lime significantly increased biomass.

Table 25. Crop residue generated and incorporated under the influence of long term INM practice with Biofertilizers

INM practices	Crop residues (Mg ha^{-1})				
	Green gram	Maize	Cabbage	Cowpea	Ragi
Absolute control	2.07	2.10	8.2	13.8	2.91
BFs	2.58	2.14	11.1	14.8	2.72
Half STD + BF	2.81	8.35	12.5	17.3	2.88
STD	1.64(-34)	4.24(-21)	13.3(-52)	26.5(-32)	3.73(2)
STD + organics	2.47	5.35	27.8	39.0	3.65
STD +Organics + BF	2.58(5)	5.55(4)	29.1(5)	42.8(10)	3.86(6)
STD +Organics + L + BF	2.66(8)	6.51(22)	30.1(8)	46.8(20)	4.00(10)
LSD (p=0.05)	0.19	0.75	2.4	3.4	0.38

The residue recycling of five crops resulted in nutrient cycling (total of five crops) of N ranging from 20 to 60 kg ha^{-1}, P from 5.2 to 16.5 kg ha^{-1}, K from 21 to 55 kg ha^{-1} and S from 2.4 to 8.7 kg ha^{-1} per crop (Table 26).

Table 26. Nutrients recycled (average per crop) due to incorporation of crop residues

INM practices	Nutrients cycled (average) per crop (kg ha^{-1})					
	N	P	K	S	Ca	Mg
Absolute control	20	5.2	21	2.4	18.9	7.5
BFs	28	5.9	27	3.7	21.5	10.3
Half STD + BF	36	7.8	34	4.4	27.0	12.3
STD	39	9.3	32	4.7	27.0	12.0
STD + organics	54	13.5	45	6.7	41.0	16.4
STD +Organics + BF	57	15.2	49	7.4	45.0	17.6
STD +Organics + L + BF	60	16.5	55	8.7	48.0	19.6
LSD (P=0.05)	7	1.0	5	0.8	4.3	1.7

Integrated management of nutrients in five different crops resulted in recovery on an average for N, P, K and S from 28 to 67 per cent, 19 to 49 per cent, 49 to 107 per cent and 13.8 to 39.2 per cent respectively. Combining organics with STD had more influence on recovery of nutrients than BFs and soil amelioration measures. However, combination of three (organics, BFs and liming) had significant influence on recovery of added nutrients (Fig 18).

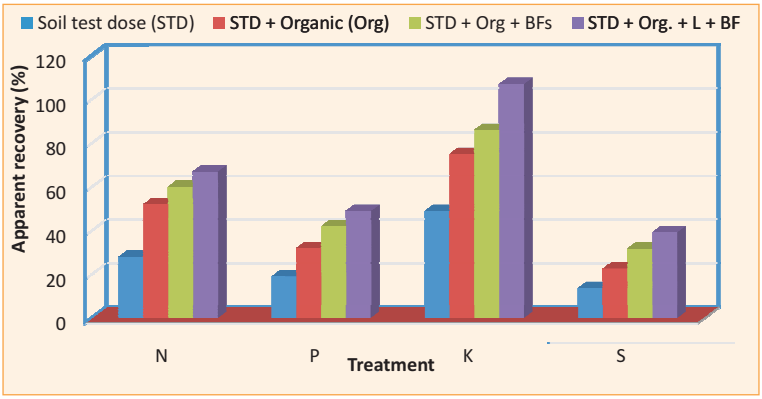
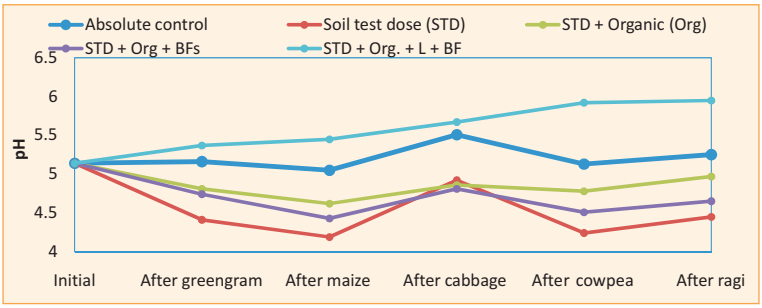


Fig 18. Apparent nutrient recoveries under the influence of different long term INM practices.

Initial soil reaction (pH) was 5.14. Except in absolute control and STD + organics + lime + BFs treated soils, the pH decreased drastically in STD. In absolute control the pH remained in the range of 5.05 to 5.25. In limed treatment the soil pH increased and ranged from 5.37 to 5.95 (Fig 19)



The initial organic carbon content was 2.7 kg^{-1}soil. Irrespective of the INM practices, the organic carbon content in soil increased, maximum being in STD + organics + Lime + BF treatment (ranging from 5.7 to 6.2 g kg^{-1}) (Fig 20).

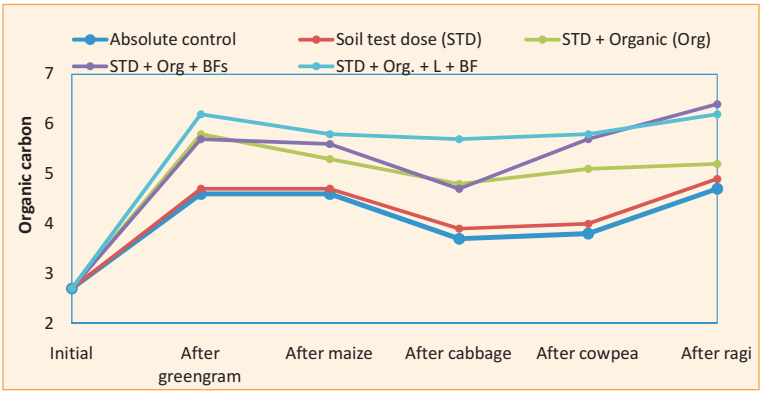


Fig 20. Change in organic carbon status over the years of different INM practices.

The monetary advantage generated out of four inputs like fertilizers (STD), organics (FYM/VC), BFs and lime for each crop indicated that for crops like cabbage and green gram, the organics contributed more than fertilizers (Table 27). The BFs component was third important contributor for economic benefits except for ragi where it was contributing only Rs.4200 ha^{-1}.

Table 27. Monetary advantages generated out of inputs used in different crops under the influence of INM practices using BFs

Inputs	Monetary advantage generated (Rs. ha^{-1})				
	Green gram	Maize	Cabbage	Cowpea	Ragi
Fertilizers (STD)	10800	50310	84500	14500	31200
Organics	13000	9360	124500	11000	6600
BFs	3750	9360	23000	1500	4200
Lime	2700	8190	22500	900	10200

Encapsulation of green gram seed with lime in acid soil

Field experiment on encapsulation of green gram seed with lime using gum acacia or sago as sticker brought significant increase in seed yield by 17 and 28 per cent respectively over the seed yield of 674 kg ha^{-1} due to side dressing with lime @ 02 LR. Such a practice increased nodular N, extra N gain and apparent recovery of P, K and S considerably (Table 28).

Table 28. Influence of lime coating of green gram seed on yield and nutrient recovery in acid soil

Treatments	Seed yield (kg ha^{-1})	Nodular N (%)	Extra N gain (kg ha^{-1})	APR (%)	AKR (%)	ASR (%)
STD + FYM+ lime (soil) + *Rhizobium* (R)	674	2.13	26	26	32	7
STD + FYM+ lime (seed) + *Rhizobium* (R)	787	2.39	35	44	52	10
R + Lime (seed) + FYM + STD	866	2.59	48	52	69	11
LSD (p = 0.05)	100	0.21	-	-	-	-

1.15. Native diazotrophs for spices (KAU, Thrissur)

Novel nitrogen fixing bacteria obtained from the rhizosphere of black pepper from Wayanad-*Microbacterium* and *Paenibacillus* were evaluated at four locations on black pepper (Fig 21) and ginger along with *Azospirillum lipoferum* (reference culture of KAU). Farmer's practice (FP) served as control. Biofertilizers were applied as soil application or mixed with FYM/ compost in August-September. Application of all the biofertilizers gave significantly higher number of lateral shoots and spikes per unit area as also spike length (except *Azospirillum*) in black pepper. However there was no significant difference in number of berries per spike, pedicel length, per plant yield, 1000 berry weight and 1000 berry volume due to application of biofertilizers. All the bio-fertilizers were superior to control (FP) with respect to plant growth parameters like number of tillers and plant height in ginger but not the number of leaves.

Fig 21. Field evaluation of novel nitrogen fixing biofertilizers on black pepper in Wayanad.

Rhizosphere soil samples were collected from different crops including cowpea, arecanut, chilli and black pepper from Wayanad area in Kerala. A total of 4 isolates of *Azospirillum,* 12 isolates of *Azotobacter* (Fig 22) and 8 of *Rhizobium* were obtained.

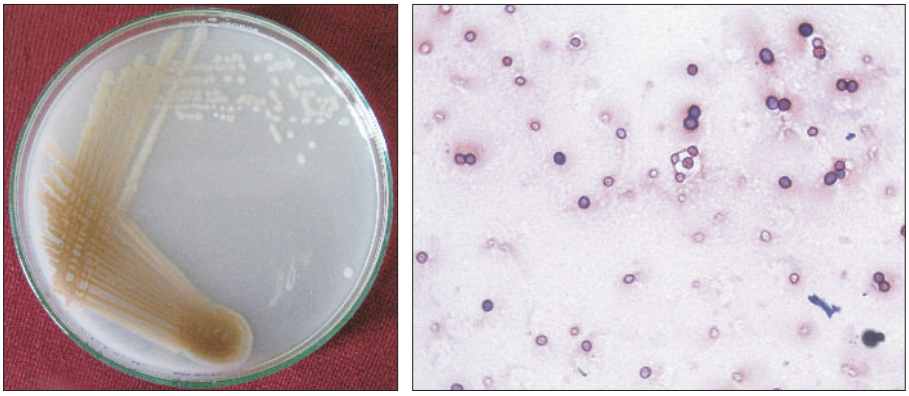


Fig 22. *Azotobacter* from Wayanad (a) pigment production on Ashby's agar, (b) older cells forming cysts.

Of the 8 cowpea nodule bacteria isolates, RH-2, RH-3, RH-5, RH-6 and RH-7 induced nodule formation in cowpea seedlings. The 16S rRNA gene sequencing revealed that RH-3, RH-5 and RH-7 belonged to genus *Rhizobium* (Table 29).

Table 29. Details of rhizobia isolated from Wayanad, Kerala

Isolate	NCBI accessions showing maximum homology		Identity %
	Accession no.	Name	
RH-1	HM798684.1	Uncultured Rhizobiales clone	96
RH-3	EU794283.1	Uncultured *Rhizobium* sp. clone EMP N5	77
RH-5	KF870446.1	*Rhizobium* sp. LS-079	94
RH-6	KJ424423.1	Uncultured bacterium clone 10C1-66	95
RH-7	KF870446.1	*Rhizobium* sp.LS-079	95
RH-8	KJ424423.1	Uncultured bacterium clone 10C1-66	95

1.16. Development of Biofertilizers for Fibre Crops (AAU)

The application of biofertilizer consortia (*Azospirillum*, *Azotobacter* and PSB) as component of INM for three years in Jute resulted the higher yield of fibre (Table 30). The pooled analysis of three years result showed that INM treatment consisted of 50% NP (15 and 13 kg ha^{-1}) and 100% K (25kg ha^{-1}) with biofertilizer consortia (*Azospirillum*, *Azotobacter* and PSB) as seed treatment produced highest (2760 kg ha^{-1}) fibre yield which was comparable with the 100% chemical fertilizers (2660 kg ha^{-1}) (Fig 23, Table 30). In the experiment it was observed that reduction of N&P fertilizer to 50% could significantly improve the soil enzyme activity.

Fig 23. Jute crop showing favourable growth with bioinoculation in Assam.

Table 30. Fibre yield of jute under integrated nutrient management

Treatments	Fibre yield (kg ha^{-1})	MBC (µg g^{-1})	FDA (µg fluorescein $g^{-1}hr^{-1}$)	DHA (µgTPF g^{-1} $24hr^{-1}$)	PHM (µg p-nitrophenol $g^{-1}hr^{-1}$)
Absolute Control	2270	702	8.2	42.5	54.7
RD of NPK	2660	832	10.9	61.9	64.3
25% RD of with 100% K+ Biofertilizer consortia	2450	818	10.4	69.1	87.1
50% RD of NP with 100% K + Biofertilizer consortia	2760	830	11.4	81.1	93.2
Biofertilizer consortia	2710	812	10.5	60.5	78.5
LSD (p=0.05)	350	21	1.3	8.8	12

RD of NPK (30:25:25); MBC: Microbial biomass carbon; DHA: Dehydrogenase; PHM: phosphomonoesterase; FDA: Fluorescein diacetate

2. Impact Assessment of Soil Management Practices on Microbial Functions and Soil Health using Genomic Tools

2.1. Analysis of structural and functional diversity of microorganisms in organic farming practices (UAS)

Structural diversity

The dominant bacterial species, protein coding genes and their relative proportion in rhizosphere soil of organically and inorganically grown (with agrochemicals) soybean (seven year old experiment) was studied by whole metagenome shotgun sequencing. The DNA of rhizosphere soil extracted at different growth stages of soybean was pooled at equimolar ratio and sequenced by semiconductor sequencing technology of Ion Torrent personal genome platform. The Q20 sequence reads generated by Torrent server were phylogenetically and functionally classified by M5NR and subsystem based classification (MG-RAST) respectively. The statistical significance in the number of reads between organic and conventional soil was calculated by Fisher's exact test using statistical analysis of metagenomic profile (STAMP). The relative proportion of *Alpha-proteobacteria* and *Actinobacteria* are high in organic soil (Fig 24) while *Gammaproteobacteria* are very high in inorganic soil.

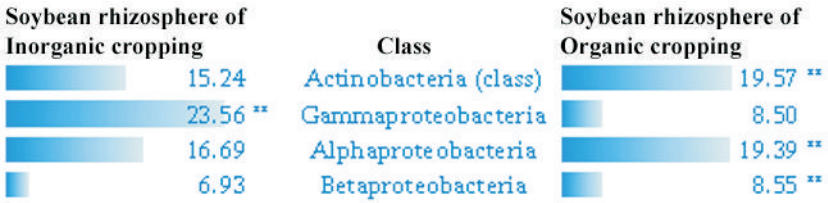


Fig 24. Relative proportion (%) of bacterial phyla in rhizosphere soil of organic and inorganic soybean. Values followed by ** are significantly more at $p \leq 0.01$ by Fisher's exact test.

Presence/absence of indicator species (beneficial/pathogenic) is another approach to define soil health. Significantly large proportion of bacteria known for beneficial role were observed in organic farm soil (Fig 25). The relative proportion of *Streptomyces, Rubrobacter, Pseudomonas Rhodopseudomonas* and *Methylobacterium* improved significantly in organic soils showing that organic farming not only improves nutrient status of soil but also improves its capacity to protect plants from pathogens.

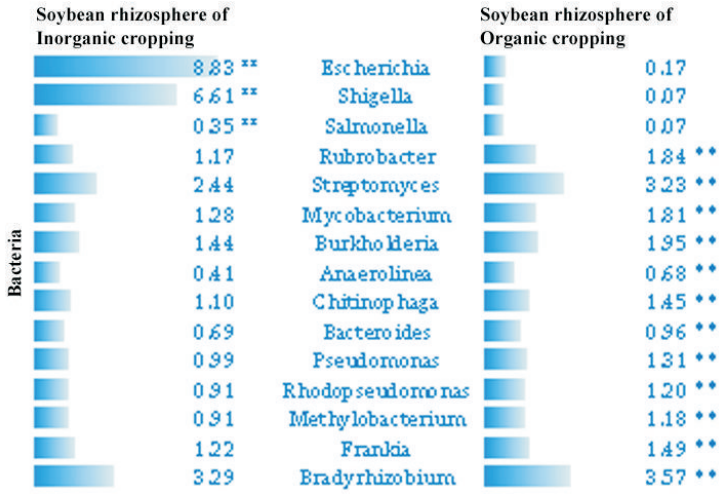


Fig 25. Relative proportion (%) of microbial genera in rhizosphere soil of organic and inorganic soybean.

Functional diversity

Metabolic activity of soil microbes can also be identified by transcription and translation process. Both organic and inorganic farm soil had more genes involved in transcription (RNA polymerase), but the relative abundance was significantly more in organic farm soil (Fig 26). More number of genes responsible for DNA repair and replication of Archeal DNA indicate that the pesticides and fertilizers could cause damage to microbial DNA in inorganic soil. On the other hand, significantly large number of genes for plasmid replication in organic farm soil is an indicative of highly active bacteria.

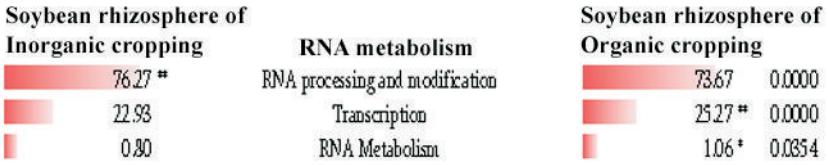


Fig 26. Relative proportion of genes (%) involved in RNA metabolism in organic and inorganic soil.

Eubacterial diversity in soils amended with different organic manures

The soil bacterial diversity associated with rhizospheric soil of maize applied with different organic fertilizers (FYM, Vermicompost, Poultry manure and Sheep manure) was investigated by PCR-DGGE and compared with recommended dose of inorganic fertilizers (RDF) and no nutrient control. The DGGE fingerprint data was converted to numerical value and the bacterial diversity was calculated by Shannon diversity index. Among the organic farming components, FYM applied soil showed highest bacterial diversity (2.35) which is similar to RDF (2.32) and no nutrient control (2.22) (Fig 27). The bacterial diversity in soil applied with other components of organic farming [Vermicompost (2.02), Poultry manure (2.02) and Sheep manure (2.07)] was similar but less than RDF and no nutrient control (Fig 27).

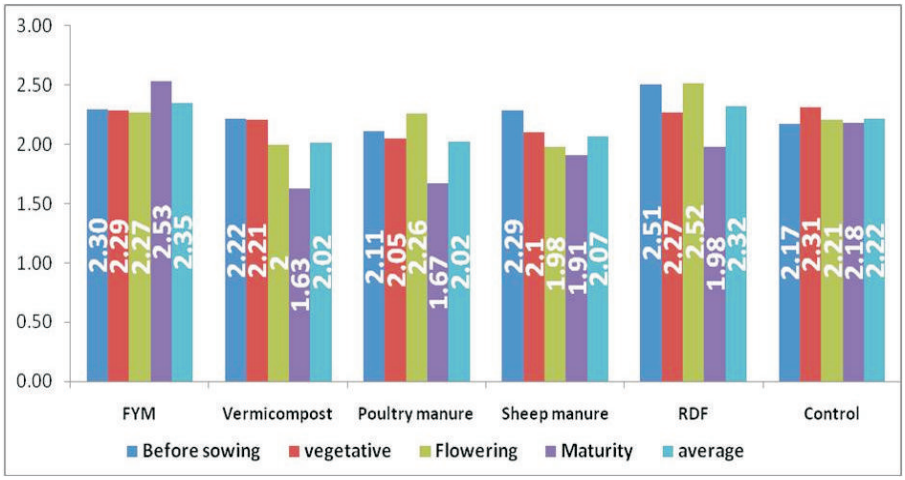


Fig 27. Shannon diversity index of the bacterial species present in rhizospheric soil at various growth stages of maize applied with organic manures/inorganic fertilizers.

2.2. *Impact of nutrient management on genes invloved in carbon sequestration processes in semi-arid tropical soils* (TNAU)

The impact of long-term nutrient management with inorganic fertilizers-IC; organic manures-OM; and integrated nutrient management- INM in the century-old permanent manurial experiment at Tamil Nadu Agricultural University, Coimbatore was assessed by analysis of soil organic carbon fractions, FTIR spectra of humic substances, soil enzyme activities and analysis of genes invloved in carbon sequestration. Fourier Transform Infrared (FTIR) spectra for the humic acid extracts of SOC were obtained at wavelengths of 4000–400 $cm^{-1.}$ The FTIR spectra did not differ noticeably between top soil and subsoil. However spectral variances were observed between nutrient treatment soils. Generally, the FTIR spectra in treatments receiving organics (FYM or INM) showed intricate pattern of bands specifically in 1640 - 1000 cm^{-1} region. This implies the complex nature of compounds of humic acid in the organically managed soil. The DNA extracted from all the soil samples were quantified for 16S rRNA gene (eubacterial abundance), *cbbL* (Rubisco, photosynthetic carbon fixing genes) and *chiA* (chitinase gene, global indicator gene for carbon cycling). Amplification was performed in real-time PCR system (Roche Lightcycler® 480II, Roche, Switzerland) by using SYBR green detection system. Two independent qPCR assays were performed for each gene and for each soil sample. Standard curve was obtained with serial dilutions of a known amount of plasmid DNA containing a fragment of the 16S rRNA, *cbbL* and *chiA* genes previously amplified from respective positive control strains of *E.coli*, *Chlorella* sp. and *Streptomyces* sp. respectively. Purity of the amplified fragment was checked by observing single melt curve at the end of qPCR run. Quantification of gene copies present in the soil samples was done and expressed as copies per g dry weight of soil.

Table 31. Impact of long term nutrient managements on abundance of 16S rRNA, *ccbL* and *chiA* genes of semi-arid tropical Alfisol

Treatments	16S rRNA gene (log_{10} copies/g soil)		*cbbL* gene (log_{10} copies/g soil)		*chiA* gene (log_{10} copies/g soil)	
	top soil (0-25 cm)	sub soil (25-50 cm)	top soil (0-25 cm)	sub soil (25-50 cm)	top soil (0-25 cm)	sub soil (25-50 cm)
Control	11.8 (±0.3)^{b}	10.6 (±0.2)^{b}	4.1 (±0.2)^{a}	2.7 (±0.5)^{a}	2.7 (±0.1)^{c}	2.4 (±0.1)^{b}
IC	12.3 (±0.1)^{ab}	10.6 (±0.4)^{b}	4.0 (±0.1)^{a}	2.8 (±0.4)^{a}	2.1 (±0.1)^{c}	2.0 (±0.1)^{b}
OM	12.6 (±0.2)^{ab}	11.4 (±0.2)^{a}	4.4 (±0.2)^{a}	2.8 (±0.5)^{a}	6.2 (±0.1)^{a}	3.2 (±0.1)^{a}
INM	12.7 (±0.1)^{a}	11.4 (±0.1)^{a}	4.7 (±0.4)^{a}	3.1(±0.8)^{a}	4.5 (±0.1)^{b}	3.1 (±0.1)^{a}

Values are mean ± SE (n=6) and within each column, values followed by same letters are not significantly different from each other according to DMRT ($p \leq 0.05$). IC - Inorganic chemical fertilized soil; OM - Organically managed soil; INM - Integrated nutrient management adopted soil.

The INM treatment showed significantly higher copies of 16S rRNA gene than those from control, IC and OC (Table 31). The abundance of green-like Rubisco gene representing Form I Rubisco of photosynthetic and chemotrophic carbon-fixing gene of the soil was measured for the first time. The abundance of this gene was in 10^4 copies per g soil and 10^2 copies in subsoil. The long-term nutrient management adoptions did not alter the abundance of this gene. With reference to chitinase gene, the long-term organic amendment adopted soils (OM and INM) had significantly higher abundance than IC and control. The IC and control samples had same trend of all the three assessed genes for both topsoil as well as for subsoil.

2.3. Cultural and metagenomic analysis of pesticide contaminated soils (DU)

Hexachlorocyclohexane contaminated soil showed enrichment of several bacteria like *Sphingomonas* and other organisms. New isolates characterised include:

1) *Pontibacter ummariensis* sp. nov., a pinkish-red, Gram negative, aerobic, rod from Ummari village, Lucknow, U.P. 16S rRNA gene sequence analysis showed that strain NKM1^T clustered with members of the genus *Pontibacter* that belongs to Cytophagaceae, phylum Bacteroidetes.

2) *Luteimonas tolerans* sp. nov., a Gram negative, aerobic, rod, yellow pigmented strain UM1^T with 16S rRNA gene sequence similarity to genus *Luteimonas* with *Luteimonas aestuarii* B9^T as the closest neighbour.

3) *Tessaracoccus flavus* sp. nov., a Gram positive, non-motile, isolated from drainage of the India Pesticides Limited (IPL), a lindane producing unit situated at Chinhat, Lucknow, U.P. 16S rRNA gene sequence analysis showed that strain RP1T belongs to the family Propionibacteriaceae and is closely related to the members of the genus *Tessaracoccus.*

Pan-genome dynamics of *Pseudomonas* gene complements

Phylogenetic heterogeneity across *Pseudomonas* genus is complemented by its diverse genome architecture enriched by accessory genetic elements (plasmids, transposons, and integrons) conferring resistance across this genus. A stress tolerant *Pseudomonas* sp. strain RL isolated from a hexachlorocyclohexane (HCH) contaminated pond (45 mg of total HCH g^{-1} sediment) was sequenced and its gene repertoire was compared with that of 17 reference ecotypes (Fig 28 and Fig 29) belonging to *P. stutzeri*, *P. mendocina*, *P. aeruginosa*, *P. psychrotolerans* and *P. denitrificans,* representing metabolically diverse ecosystems (i.e. marine, clinical, and soil/sludge). Metagenomic data from HCH contaminated pond sediment and similar HCH contaminated sites were further used to analyze the pan-genome dynamics of Pseudomonas genotypes enriched across increasing HCH gradient. Although strain RL demonstrated clear species demarcation (ANI $\leq$ 80.03%) from the rest of its phylogenetic relatives, it was found to be closest to P. stutzeri clade which was further complemented functionally. Comparative functional analysis elucidated strain specific enrichment of metabolic pathways like α-linoleic acid degradation and carbazole degradation in Pseudomonas sp. strain RL and P. stutzeri XLDN-R, respectively. Composition based methods (% codon bias and % G+C difference) further highlighted the significance of horizontal gene transfer (HGT) in evolution of nitrogen metabolism, two-component system (TCS) and methionine metabolism across the Pseudomonas genomes used in this study. An intact mobile class-I integron (3,552 bp) with a captured gene cassette encoding for dihydrofolate reductase (*dhfra1*) was detected in strain RL, distinctly demarcated from other integron harboring species (i.e. *P. aeruginosa*, *P. stutzeri,* and *P. putida*). Mobility of this integron was confirmed by its association with Tnp21-like transposon (95% identity) suggesting stress specific mobilization across HCH contaminated sites. Metagenomic data from pond sediment and recently surveyed HCH adulterated soils revealed the *in situ* enrichment of integron associated transposase gene (TnpA6100) across increasing HCH contamination (0.7 to 450 mg HCH g^{-1} of soil). Unlocking the potential of comparative genomics supplemented with metagenomics, this study attempted to resolve the environment and strain specific demarcations across 18 *Pseudomonas* gene complements. Pan-genome analyses of these strains indicated astoundingly diverse metabolic strategies and provide genetic basis for the cosmopolitan existence of this taxon which can be exploited to mine organisms for targeted bioremediation.

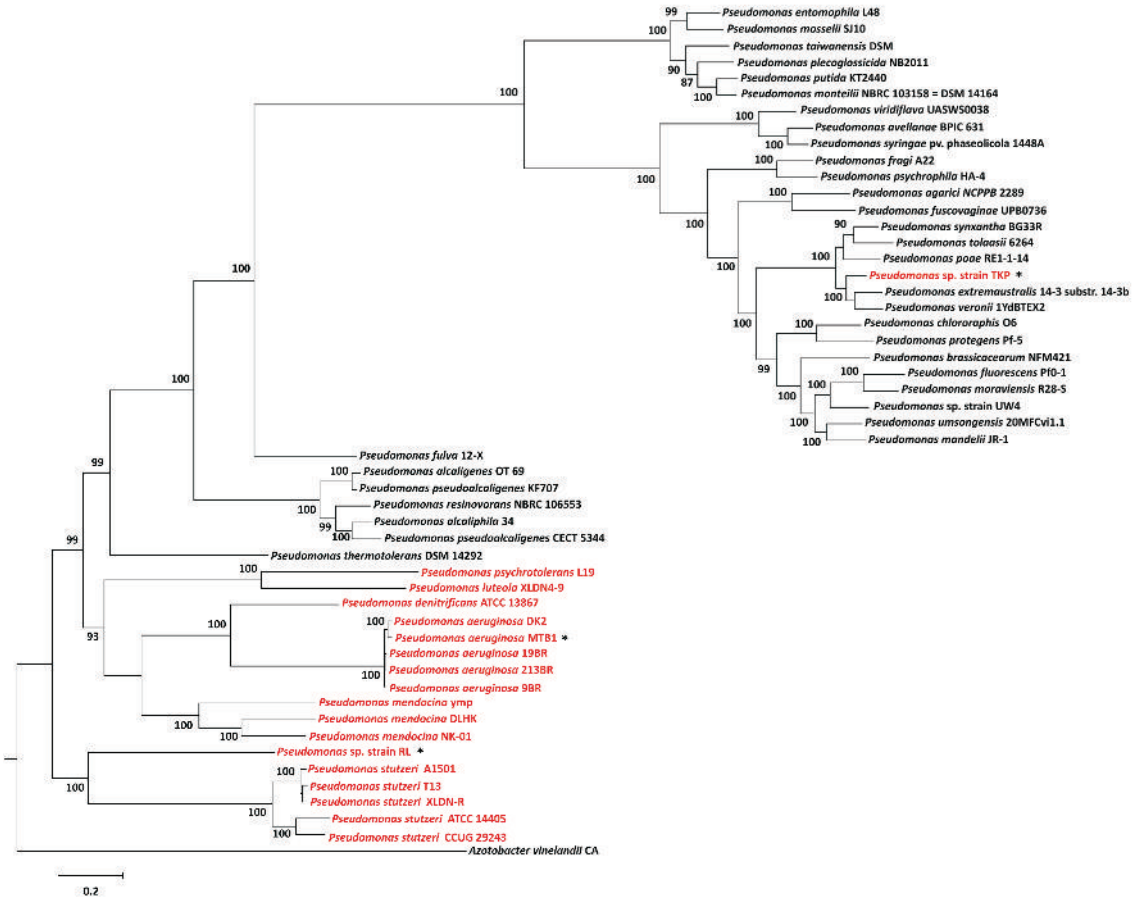
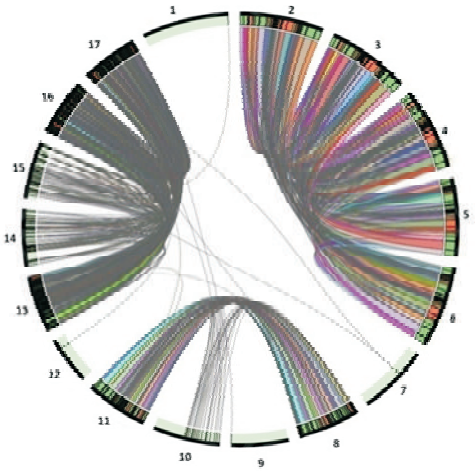


Fig 28. Phylogenomic analysis of *Pseudomonas* genotypes: Conserved genes (400) based phylogenetic tree of strain RL and representative *Pseudomonas* genotypes (n=51) constructed at 1000 bootstrap with *Azotobacter vinelandii* as an out-group. Strains labeled in red color are the ones chosen for the comparative study and those labeled with asterisks (*) are the ones inhabiting HCH contaminated soils. Branch lengths are drawn to scale, with scale bar indicating the number of amino acid substitutions. Numbers on branches are the bootstrap values of the clusters on the right.



1. ***Pseudomonas* sp. strainTKP**
2. ***Pseudomonas aeruginosa* 98R**
3. ***Pseudomonas aeruginosa* 198R**
4. ***Pseudomonas aeruginosa* 2138R**
5. ***Pseudomonas aeruginosa* DK2**
6. ***Pseudomonas aeruginosa* MTB-1**
7. ***Pseudomonas denitrificans* ATCC 13867**
8. ***Pseudomonas mendocina* DLHK**
9. ***Pseudomonas luteola* XLDN4-9**
10. ***Pseudomonas mendocina* NK-01**
11. ***Pseudomonas mendocina* ymp**
12. ***Pseudomonas sp. strain* RL**
13. ***Pseudomonas stutzeri* A1501**
14. ***Pseudomonas stutzeri* ATCC 14405**
15. ***Pseudomonas stutzeri* CCUG 29243**
16. ***Pseudomonas stutzeri* T13**
17. ***Pseudomonas stutzeri* XLDN-R**

Fig 29. Whole genome synteny plot of *Pseudomonas* sp. RL and its 17 phylogenetic neighbors. A window size of 5 kb was used. Colored blocks (red/green) for each genome's base represents their orientation; green=positive, red=negative. Connecting arcs are drawn in grey color.

2.4 Split-agar assay of antifungal soil microbial metabolites (IISS)

Soil microorganisms suppress soil borne plant pathogenic fungi through various mechanisms. There is no appropriate, integrated method to easily quantify soil health in terms of disease control ability. A novel assay to quantify the ability of soil to inhibit fungal pathogens was devised. The technique is easy to use routinely in investigations for soil biological quality testing as it offers a quantitative and integrated expression of suppressiveness as actidione equivalents per gram of soil. Soil samples were inoculated into liquid growth medium and incubated; supernatants were filter sterilized and assayed in split agar (Fig 30) against *Macrophomina phaseolina* to record colony radius. The antifungal activity of the soils varied widely ranging from 0.02 to 2.80 mg actidione equivalents g^{-1} soil, of which 81–98% was heat labile. The test will be a useful aid in decision support for reducing the use of chemical control agents and promote sustainable farming practices.

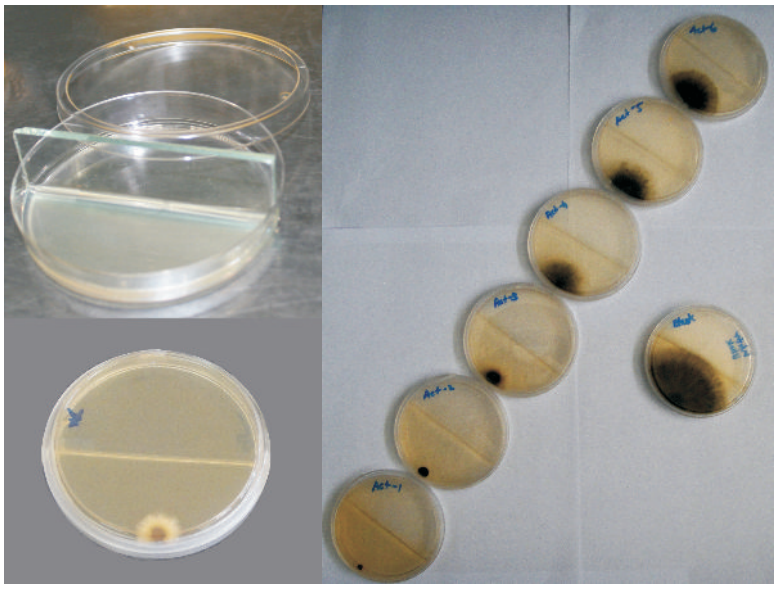


Fig 30. Steps in preparation of split-agar plates

a) Sabouraud's dextrose medium with 1 % agar in half plate bounded by glass slide.

b) Plate with soil suspension incorporated Sabouraud's agar in second half showing inhibition of *M. phaseolina* inoculated on first half of the plate.

c) Plates with actidione incorporated Sabouraud's agar in second half, showing linear response of growth inhibition of *M. phaseolina* (inverse of colony radius) to increasing actidione concentration.

3. Improvement of Biofertilizer technology for high quality and improved delivery

3.1. Refinement and Testing of Liquid Biofertilizer Technology (ANGRAU)

Black gram

By using the liquid biofertilizer technology developed by this ICAR project the farmers in Andhra Pradesh are saving 20-25% of chemical fertilizers and reporting 10-15 % additional yields in their crops. Often farmers miss the usage of biofertilizers at the time of sowing crops due to several reasons including labour cost and the need to keep them separate and not mix with chemical fertilizers or agro-chemicals. To overcome this problem post application of biofertilizers on blackgram at early stage of the crop was done by using Taiwan sprayer. About 70-100 litres of water were mixed with specified quantity of liquid N and P biofertilizers (*Rhizobium* and PSB) mixed vigorously and applied on the field surface within 15 days after germination of the crop (Fig 31). This method gave crop performance at par with the solid biofertilizers applied at the time of sowing (Table 32) showing that if a farmer is missing the application of biofertilizers at the time of sowing the crop can be saved by applying liquid biofertilizers instead of solid biofertilizers within fifteen days after sowing to obtain good crop yield.

Fig 31. Post application of liquid biofertilizers in blackgram field by using Taiwan sprayer.

Table 32. Effect of time of application of Biofertilizer formulations on Blackgram crop

Treatment	Nodule no. plant^{-1}	Nodule DM (mg plant^{-1})	Shoot DM (Mg ha^{-1})	Grain yield (kg ha^{-1})
Absolute control	16	228	3.9	746
Solid Biofertilizers at Sowing	64	870	5.8	1426
Solid Biofertilizers Post Sowing	45	634	4.7	1145
Liquid Biofertilizers at Sowing	80	1165	6.4	1567
Liquid Biofertilizers Post sowing	72	1071	6.1	1431
LSD (p=0.05)	11	109	1.8	158

The influence of humic acids and amino acids collected from different sources was studied on blackgram. The results indicated that mixed microbial consortium gave the best grain yields which was much higher than humic acid application (Table 33).

Table 33. Influence of humic acid and microbial inoculants on Blackgram

Treatment	Grain wt. (Kg ha^{-1})
Control	279
Biofertilizers (*Rhizobium*+PSB)	474
Fish Amino acids+Biofertilizers (*Rhizobium* +PSB)	489
Humic acid commercial + Biofertilizers(*Rhizobium* +PSB)	517
Humic acid (Vermicompost) + Biofertilizers(*Rhizobium* +PSB)	594
Rhizobium+PSB+PGPR+VAM	740
100 % RDF	602
LSD (p=0.05)	87

Pigeonpea

Three pigeonpea rhizobial isolates PPR 701 (slow grower) and PPR 704 and PPR 808 (fast growers) (Table 34) are widely used in production of rhizobial inoculants for pigeonpea at ARS, Amaravathi.

Table 34. Effective pigeonpea rhizobial strains developed at ARS, Amaravathi

Code	Isolated from	Place	Growth rate	pH of media
PPR 701	Vertisol	Patancheru, Medak Dt. Telangana	Slow	7.0-7.6
PPR 704	Vertisol	Nidumukkala, Guntur Dt., A.P.	Fast	7.0-7.4
PPR 808	Alfisol	Samalkot, East Godavari Dt., A.P.	Fast	6.8-7.2

During the year 2015-16 severe drought conditions prevailed after sowing of the crop. This situation helped in evaluating the performance of different formulations under drought stress in Alfisol. Liquid biofertilizers enhanced the crop growth even under 45% deficit rainfall by improving nodulation by 20% (Table 35).

Table 35. Effect of biofertilizers formulations under drought stress situation on pigeon pea

Treatment	Soil pH	Nodule no. pl^{-1}	Nodule DM (mg pl^{-1})	GrainYield (kg ha^{-1})
LBF(*Rhizobium*+PSB)+OM	7.6	57	848	1520
PBF(*Rhizobium*+PSB)+OM	7.7	42	690	1461
50%RDF+ LBF(*Rhizobium*+PSB)+OM	7.5	78	1206	1776
50%RDF+ PBF(*Rhizobium*+PSB)+OM	7.8	65	1048	1527
100% RDF	7.7	30	550	1453
Absolute control	8.0	32	561	1150
LSD (p=0.05)	-	8	90	176

Potassium solubilising bacteria influence on Sorghum

Five efficient bacterial strains for releasing potassium from the bounded mineral sources (KRB 103,105, 111, 114 and 115) were isolated and characterized. Clearing zones (Fig 32) varied from 16-22 mm and K solubilisation index varied from 3.1-6.3 with maximum shown by KRB 111 isolate.

This isolate performed well in field on sorghum and plants showed early panicle initiation and grain maturity when compare to sole chemical fertilizer applied plots (Fig 32) The treatment of 75% RDF+ KRB+NFB+PSB gave significantly higher grain yields over 100% RDF (Table 36).

Fig 32. Potassium releasing bacterial isolate KSB 111 and inoculated Sorghum crop.

Table 36. Influence of Potassium releasing bacterium (KRB) on sorghum crop

Treatment	pH	Organic C (%)	Available K (kg ha^{-1})	Grain yield (kg ha^{-1})
Control	7.6	0.48	398	1575
25% RDF + KRB	7.3	0.51	489	1629
50%RDF+KRB	7.2	0.55	507	1678
75% RDF+KRB	6.9	0.53	512	1760
25% RDF+KRB+NFB+PSB	7.1	0.52	544	1688
50% RDF+KRB+NFB+PSB	7.0	0.54	553	1854
75% RDF+KRB+NFB+PSB	6.9	0.56	556	2125
100% RDF	7.3	0.50	503	1897
LSD (p=0.05)	-	NS	129	220

Rapid Decomposition of Agricultural Wastes

By using both fungal and bacterial cultures a microbial consortium for rapid decomposition of agricultural wastes within a period of 50-60 days was developed and is being manufactured and supplied to farmers for rapid decomposition of all agricultural wastes (Fig 33).

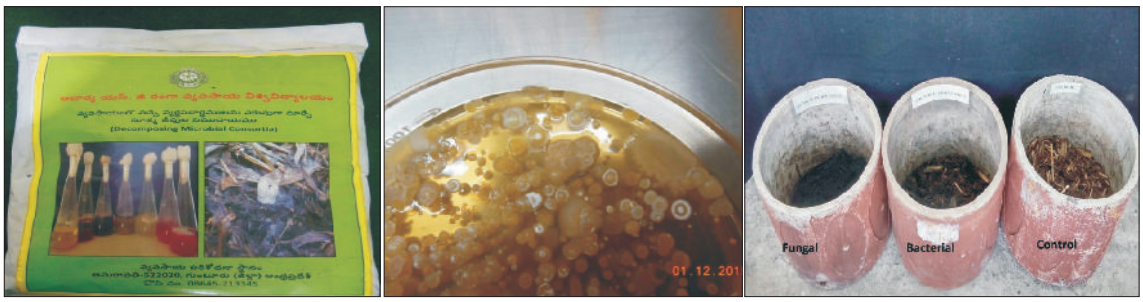


Fig 33. Inoculant for decomposition of agricultural wastes (L), Decomposing Microbial consortium (M) and State of decomposition with microbial inoculants (R).

3.2. Biofertilizer technology for VA-Mycorrhiza

***Probing clues for triggering in vitro growth of AM fungi* (CRURRS)**

AM- fungi are inoculated and multiplied in the rhizosphere of susceptible crops and the infected root material along with rhizosphere soils are then used as inoculum. Artificial culturing of AM-fungi has proven difficult because of lack of knowledge about what compounds can trigger their growth on synthetic medium. Several AM-fungal growth stimulatory compounds under natural system like strigolactones, flavonoids, triglycerides present in root exudates have been reported. MtPT4 andMtPT6 are phosphate transporter genes expression products (proteins) present in roots of *Medicago truncatula* which play important role in Pi transport during AM symbiosis. These proteins were also reported to be produced by AMF. It has been hypothesised that the stimulatory compounds, present on plant root exudates, could be bio-activated by binding with the receptor proteins like MtPT4 and MtPT6 provided the latter have receptor sites for them. So, attempts were made to optimize these receptor proteins using bioinformatic tools by molecular docking with the aid of 'Accelyrs Discovery Studio 2.5' software. The constructed 3-dimentional structure of MtPT6 (using PDB SUM), in-silico homology modeling & docking studies against bioactive compound (triglycerides) stimulating AM-fungal growth revealed the presence of favourable receptor sites (Fig 34). If these receptor proteins could be successfully bound with triglycerides under wet-lab conditions and rendered stable in a synthetic medium, then they can be used to promote AM fungal growth in absence of living root system.

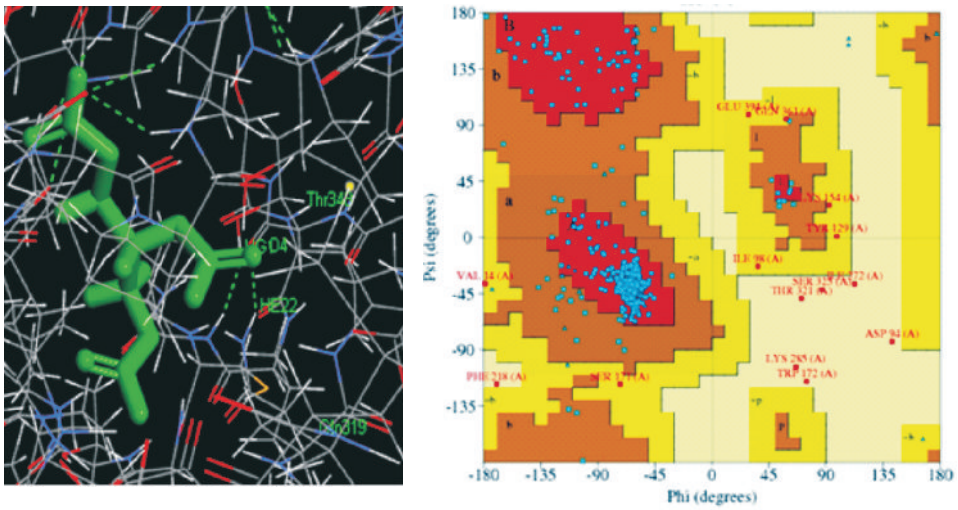


Fig 34. Triglycerides CID: 5460048 docked with MtPT6 receptor protein (left); Validation of structure of MtPT6 generated through Ramachandran plot.

Easy and rapid *in vitro* production technology for AM fungi (ANGRAU)

Six AM fungal isolates AM-1 to AM-3 and AM-6 all belonging to *Glomus* spp. AM3, AM4 and AM5 belonging to *Gigaspora, Sclerocystis and Acaulospora* respectively, collected across different agro-climatic zones of Andhra Pradesh were multiplied on suitable host plants. About 9.7 metric tons of AM fungal biofertilizer was produced by using cotton as a host crop under semi-sterile controlled environmental conditions by using this polyhouse technology (Fig 35).

Fig 35. Production of VAM Biofertilizer using cotton as a host crop.

3.3. Seed coat formulation of bioinoculants for pulses (TNAU)

The survival of inoculant strains viz., *Azospirillum* (for maize), *Rhizobium* (for soybean), phosphobacteria and *Pseudomonas* on the seeds of maize and soybean was assessed with or without sticking agent carboxy methyl cellulose (0.1%). The microbial coated seeds were packed in polythene containers and stored at room temperature. At periodic intervals, the numbers of viable bacterial cells on the seeds were counted in specific medium. Among the three bioinoculants, *Azospirillum* and phosphobacteria on maize seed; *Rhizobium* and Phosphobacteria on soybean seed coated with carboxy methyl cellulose had longer survival (10^3 cfu/seed upto 3 months) while *Pseudomonas* population was below detectable limit (less than 100 cfu) after 60 days of storage at room temperature (Fig 36). The coating of carboxy methyl cellulose prolonged the survival, while without sticker, the survival of these organisms on the seeds of maize and soybean became below detectable limit (<100 cfu/seed) after 45 days. As the CMC coating results in seed clumping during storage, an improved semi-synthetic cellulose polymer (hydroxy propyl methyl cellulose) is under assessment.

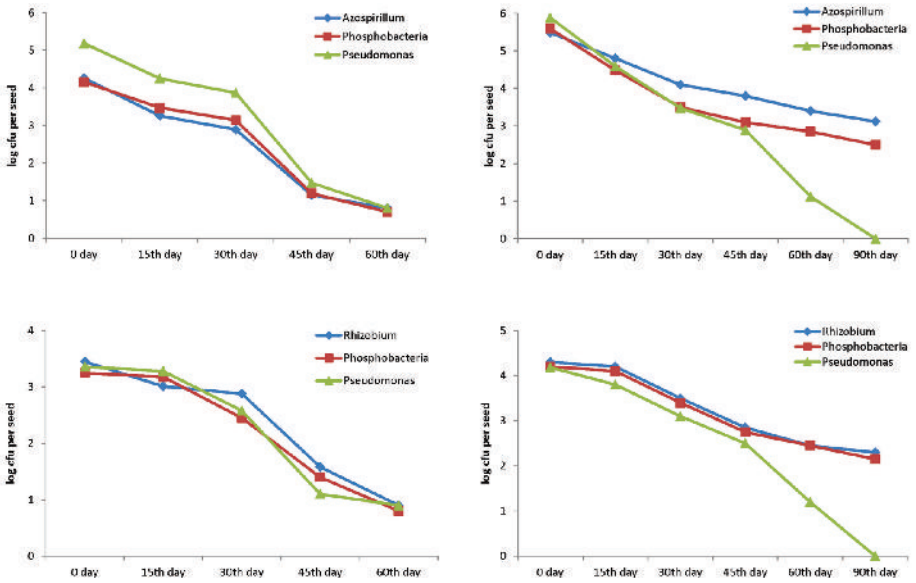


Fig 36. Survival pattern of inoculant strains on maize (top) and soybean (bottom) seeds with (right) and without (left) CMC sticker.

4. Diversification of Biofertilizer Research and Application in Drylands, Degraded soils, Tribal areas and NEH region

4.1. Evaluation of Zinc solubilizing microbial strains for field crops in Vertisols (MAU)

Soybean

On-farm field trials were conducted for three years from 2013 to 2015 to explore the potential of various microorganisms (*Bacillus megaterium*, *Pseudomonas striata*, *Trichoderma viride* and *T. harzianum* selected for their ability to solubilize zinc sulphide in media) to improve the availability of soil zinc and promote plant growth. In soybean the number of nodules/plant, dry nodule weight were significantly higher in treatment receiving inoculation of RDF + *Rhizobium* + *Trichoderma viride* over rest of the treatments (Table 37). These inoculants also increased the grain and straw yield of soybean (8.4 and 35.6 %) over fertilized un-inoculated control. Uptake of nutrients was improved with most treatments.

Table 37. Yield, nutrient uptake and quality of soybean inoculated with zinc solubilizing microorganisms

Parameters	Fertilized/ Uninoculated	*Rhizobium* + *Bacillus megaterium*	*Rhizobium* + *Pseudomonas striata*	*Rhizobium* + *Trichoderma viride*	*Rhizobium* + *Trichoderma harzianum*	LSD (P=0.05)
No. of nodules/plant	15	24	28	30	25	8
Nodules DM (mg plant^{-1})	122	177	210	213	192	41
Grain yield (kg ha^{-1})	1546	1500	1684	1830	1672	148
Straw yield (kg ha^{-1})	2003	2268	2405	2716	2560	270
Grain N uptake (kg ha^{-1})	81.1	78.8	90.0	104.4	91.4	N.S.
Straw N uptake (kg ha^{-1})	16.6	18.2	21.3	24.9	21.3	3.2
Grain P uptake (kg ha^{-1})	6.8	10.0	9.6	7.6	5.6	2.1
Straw P uptake (kg ha^{-1})	7.3	12.3	11.4	8.8	5.7	1.9
Grain Zn uptake (g ha^{-1})	82.8	76.6	85.3	105.2	87.2	NS
Straw Zn uptake (g ha^{-1})	56.9	68.4	78.3	91.4	76.3	12.4
Grain protein (%)	28.8	30.4	31.2	33.3	30.3	0.8
Grain oil (%)	16.1	15.9	16.2	16.9	16.0	0.4

Groundnut

In a field on summer groundnut in a farmer's field (Fig 37) in village Kehal in Jintur Taluka of District Parbhani in Maharashtra, zinc solubilizing cultures gave significant impact on nodulation and yield of groundnut (Table 38).

Fig 37. Harvesting of field trial of summer groundnut.

Table 38. Yield, nutrient uptake and quality of groundnut inoculated with zinc solubilizing microorganisms

	Fertilized Uninoculated	*Rhizobium* + *Bacillus megaterium*	*Rhizobium* + *Pseudomonas striata*	*Rhizobium* + *Trichoderma viride*	*Rhizobium* + *Trichoderma harzianum*	LSD (P=0.05)
No. of nodules/plant	59	107	127	122	123	4
Nodule DM mg pl^{-1}	0.78	1.26	1.58	1.52	1.55	0.06
Dry pod yield (kg ha^{-1})	2350	2596	2910	2860	2783	188
Dry haulm yield (kg ha^{-1})	3073	3219	3633	3583	3506	261
Total N uptake (kg ha^{-1})	141	154	198	191	185	11.3
Total P uptake (kg ha^{-1})	13.8	34.0	29.5	26.9	28.2	2.0
Total Zn uptake (g ha^{-1})	194.7	212.9	291.1	271.8	245.3	16.7
Kernel protein (%)	26.5	28.6	29.4	29.2	28.1	0.6
Kernel oil (%)	39.7	40.7	44.8	43.3	42.2	3.8

Cotton

There was a significant increase in the yield of seed cotton and dry matter due to inoculation of zinc solubilizing microbial cultures along with recommended dose of fertilizer (NPK) as compared to the un-inoculated control (Table 39). The magnitude of increase in Zn uptake in seed and stalk with Zn solubilizing *Trichoderma viride* and RDF was 52 and 63% over only RDF.

Table 39. Yield and zinc uptake of cotton inoculated with zinc solubilizing microorganisms

Treatments	Seed cotton yield (kg ha^{-1})	DM yield (kg ha^{-1})	Seed Zn (g ha^{-1})	Stalk Zn (g ha^{-1})
RDF	1434	2386	53.3	78.4
RDF + *Burkholderia cepacia*	1660	2844	65.5	101.0
RDF + *Burkholderia cenocepacia*	1567	2596	61.4	91.9
RDF + *Pseudomonas fluorescens*	1638	2887	66.5	105.7
RDF + *Pseudomonas striata*	1780	3171	76.0	122.2
RDF + *Trichoderma viride*	1856	3261	79.2	127.2
RDF + *Trichoderma harzianum*	1602	2809	64.1	103.6
RDF + *Bacillus megaterium*	1666	3021	69.0	115.4
LSD (p=0.05)	98.4	345	6.7	17.1

The content of DTPA Zn in soil showed significant increase under all microbial inoculated treatments compared to control treatment (Table 40). Significantly greater values were recorded with RDF + *Trichoderma viride* which was found to be at par with *Pseudomonas striata*, *Bacillus megaterium* and *Pseudomonas fluorescens*.

Table 40. Effect of zinc solubilizing microbial inoculants on DTPA extractable Zn in soil (mg kg^{-1}) in *Bt* cotton (Three years pooled mean)

Treatments	DTPA soil Zn (mg kg^{-1})			
	30 DAS	60 DAS	90 DAS	120 DAS
RDF	0.53	0.55	0.63	0.60
RDF + *Burkholderia cepacia*	0.56	0.58	0.69	0.68
RDF + *Burkholderia cenocepacia*	0.55	0.57	0.68	0.66
RDF + *Pseudomonas fluorescens*	0.58	0.59	0.72	0.69
RDF + *Pseudomonas striata*	0.59	0.63	0.73	0.73
RDF + *Trichoderma viride*	0.59	0.63	0.74	0.73
RDF + *Trichoderma harzianum*	0.56	0.60	0.70	0.68
RDF + *Bacillus megaterium*	0.58	0.63	0.73	0.72
LSD (p=0.05)	0.02	0.03	0.03	0.03
Initial DTPA soil Zn		0.52		

4.2. Evaluation of zinc solubilising microbial strains for rice in NEH region (AAU)

Inoculation of five zinc solubilizing bacteria isolated from rice and *toria* rhizosphere were compared with application of $ZnSO_4$ (25 kg ha^{-1}). $ZSBS_1$ from rice rhizosphere could significantly improve the concentration of Zn (43.1 mg kg^{-1}) in rice grain compared to application of $ZnSO_4$ (39.2 mg kg^{-1}) (Table 41). Compared to the control, significantly higher concentration of DTPA-Zn was observed in soils of other treatments (Table 41).

Table 41. Yield of Rice (var: *Ranjit*) and Zn uptake in grain and soil zinc status as influenced by Zn solubilizing bacteria

Treatments	Rice yield (Mg ha^{-1})	Zn in grain (mg kg^{-1})	DTPA-Zn in soil (mg kg^{-1})
RD of NPK(40:20:20 kg ha^{-1})	3.80	17.6	0.23
RD of NPK+ $ZSBN_2$	4.01	33.4	0.96
RD of NPK+ $ZSBK_1$	3.66	34.7	1.42
RD of NPK+ $ZSBT_1$	3.86	36.4	1.33
RD of NPK+ $ZSBS_1$	3.80	43.1	0.51
RD of NPK+$ZnSO_4$ @ 25kg/ha	4.26	39.2	1.75
RD of NPK+ $ZSBM_1$	4.15	36.6	1.29
LSD (p=0.05)	0.35	1.1	0.48

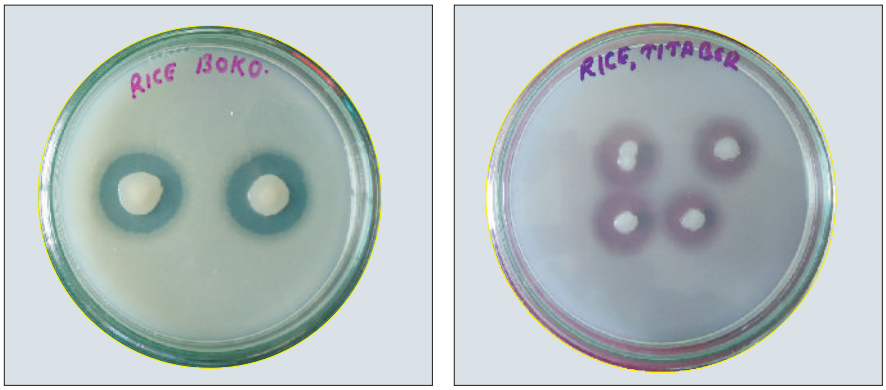


Fig 38. Zn solubilizing bacteria isolated from rice rhizosphere.

Two isolates ($ZSBS_1$ and $ZSBT_1$, Fig 38) were further tested for Zn nutrition in three widely grown varieties of rice (Bahadur, Ranjit and aromatic Joha) in Assam. Results showed that they could increase the Zn concentration (39.2 and 38.4 mg kg^{-1} respectively) in rice grain which was comparable with the application of $ZnSO_4$ (41.7 mg kg^{-1}). Among the varieties, significantly highest Zn concentration (36.9 mg kg^{-1}) was recorded in Ranjit. Compared to the control, the DTPA-Zn after harvest of rice was significantly higher either in inoculation or application of $ZnSO_4$ (Table 42).

Table 42. Effect of Zn solubilizing bacteria on Zn concentration in rice varieties

Treatments	Rice yield (Mg ha^{-1})	Zn in grain (mg kg^{-1})	DTPA-Zn in soil (mg kg^{-1})
RD of NPK (40:20:20 kg ha^{-1})	3.05	23.1	0.68
RD of NPK +$ZnSO_4$ (25kg ha^{-1})	3.58	41.7	1.25
RD of NPK + $ZSBS_1$	3.47	39.2	1.13
RD of NPK + $ZSBK_1$	3.53	38.4	1.11
LSD (p=0.05)	0.39	2.4	0.15
Variety:Bahadur	3.17	36.9	1.03
Variety:Ranjit	4.05	36.7	1.08
Variety:Aromatic joha	3.00	33.2	1.01
LSD(p=0.05)	0.34	2.1	NS

4.3. Exploitation of soil microfauna for sustainable cropping (AAU)

Collembolans extracted from soils of diverse habitats were reared on different base materials under laboratory conditions. The highest numbers were found in forest ecosystems followed by fallow land and least were in agroecosystem (Table 43). Live collembolans were separated and reared in different base materials (Table 44). One hundred collembolans were released in each of the base materials (Fig 39). Among all base materials, the survival rate of collembolans after 30 days was highest in FYM (84 %) followed by activated charcoal mixed with plaster of paris and water (78 %).

Table 43. Collembolans (no. m^{-2}) extracted from various ecosystems during (Aug 2014-Jan 2015)

Ecosystems	Months						Average	Total
	Aug	Sept	Oct	Nov	Dec	Jan		
Agroecosystem	621	837	454	215	201	90	403	2418
Fallow land	751	999	510	373	352	207	532	3192
Forest	1136	1148	913	828	511	247	797	4783

Fig 39. Rearing of collembolans in base materials.

Table 44. Survival rate of collembolans in different base materials

Treatments	Survival rate (%)		
	Day 5	Day 15	Day 30
Farm yard manure (FYM)	92.3	88.8	84.3
Vermicompost	54.8	38.8	31.8
Consortia of biofertilizers	66.0	52.8	43.0
Moist soil + leaf litter	82.0	75.5	69.8
Activated charcoal + plaster of paris + water	86.8	84.3	78.8
LSD (p=0.05)	3.9	2.5	2.4

4.4. Development of microbially enriched compost for INM and organic farming in NE India (AAU)

Evaluation and Demonstration of Biofertilizer Package for Hot Chilli

Application of microbially enriched compost @ 5 and 10t/ha over four year period showed that the average yields of hot chilli were 8 and 20% respectively higher over normal compost + Biofertilizer mixed together and applied at the time of planting (Table 45). The quality parameters ascorbic acid and capsaicin content of fruits were better over unmanured control. The soil biological as well as the chemical properties also improved on application of organic inputs (Table 45). The on-farm trials (OFT) with application of EC (10 t ha^{-1}) at three sites showed an average fresh yield of hot chilli of 2110 kg ha^{-1} (Fig 40).

Table 45: Fresh yield of Hot Chilli and Soil Biochemical Properties (mean of four years)

Treatments	Fresh yield (kg ha^{-1})	Ascorbic acid (mg 100g^{-1})	Capsaicin (%)	DHA (µg TPF g^{-1} soil 24h^{-1})	MBC (µg g^{-1})	OC (g kg^{-1})
Control	980	107.8	2.49	90.3	117.1	8.7
EC(5t/ha)	1770	116.2	2.54	129.2	174.4	9.2
EC(10t/ha)	2180	119.4	2.56	204.2	192.6	11.0
Compost(5t ha^{-1}) + Biofertilizer	1630	117.1	2.47	157.2	202.7	10.9
Compost(10t ha^{-1}) + Biofertilizer	1810	117.8	2.52	181.8	206.5	10.2
LSD(p=0.05)	150	4.7	NS	16.3	14.8	0.7

Fig 40. On farm trial of Hot Chilli in farmers field .

After four years of the organic trial, application of compost (10 t ha^{-1}) with biofertilizer showed significantly (P <0.05) higher levels of mineralized C (240.3 mg kg^{-1}) and potentially mineralizable C (327.9 mg kg^{-1}) than control (140.7 and 178.1 mg kg^{-1}respectively) at 120 days of incubation. However, the application of enriched compost (EC) (10 t ha^{-1}), produced the highest cumulative mineral N (27.5 mg kg^{-1}) followed closely by application of compost (10 t ha^{-1}) with biofertilizers (25.7 mg kg^{-1}) (Fig 41). The potentially mineralizable N was highest (53.7 mg kg^{-1}) under the treatment receiving EC (10 t ha^{-1}) for four consecutive years and significantly greater over the other treatments (Fig 41).

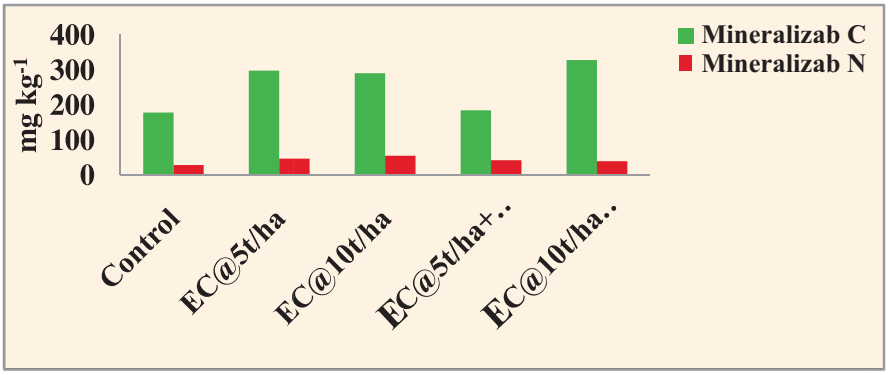


Fig 41. Mineralizable C and N under the organic inputs.

Development and Demonstration of Organic Package for Rice

The results of a five year experiment on rice showed significant yield improvement and soil organic carbon and available nitrogen with organic package (compost, enriched compost, *Azolla* dual culture and biofertilizers) (Table 46). The application of enriched compost (5 t ha^{-1}) and *Azolla* (0.5 t ha^{-1}) with biofertilizers were demonstrated as on- farm trials at four Krishi Vigyan Kendras (KVK).

The average yield of rice was 3.53 t ha^{-1} (aromatic *joha* rice) in KVK Golaghat and KVK Jorhat (Fig 42) with application of enriched compost (5.0 t ha^{-1}) compared to 3.0 t ha^{-1} in farmers practice (22.0 kg urea and 37.0 kg DAP per ha). In hilly area (KVK, Diphu) the application of enriched compost (5.0 t ha^{-1}) could produce an average yield of 4.20 t ha^{-1} (*Var*: Gaya) compared to 3.09 t ha^{-1} in farmers practice (FYM 2.0 t ha^{-1} + Urea top dressing). In KVK Lakhimpur, the application of enriched compost (5.0 t ha^{-1}) resulted 3.60 t ha^{-1} of rice yield (*Var:* Solpona) compared to 2.40 t ha^{-1} in farmers practice (FYM @2.5 t ha^{-1}). In KVK Karimganj, the application of *Azolla* (0.5 t ha^{-1}) with biofertilizers could result 4.75 t ha^{-1} of paddy yield (*Var*: Ranjit) compared to 4.40 t ha^{-1} in farmers practice (Urea 22.0 kg ha^{-1}).

Table 46. Effect of organic inputs on rice yield and soil biochemical parameters

Treatments	Rice Yield (t ha^{-1})	Av.N (kg ha^{-1})	OC (g kg^{-1})	MBC (µg g^{-1})	DHA (µgTPFg^{-1} $24hr^{-1}$)	FDA (µg fluoresce in g^{-1} h^{-1})	DTPA-Zn (mg kg^{-1})
Absolute Control	2.4	285	8.4	154.7	126.3	9.2	0.51
Biofertilizer	2.9	359	10.6	178.7	136.3	9.7	0.78
Compost @5t/ha	3.1	371	10.5	215.1	170.4	11.2	0.97
Compost @5t/ha+ Biofertilizer	3.3	366	10.1	234.6	172.1	12.2	0.77
EC @2.5/ha	2.9	370	10.9	197.7	160.0	10.2	0.66
EC @5.0/ha	3.4	398	10.2	232.9	172.8	10.7	0.71
Azolla @ 0.5t/ha +Biofertilizer	3.4	373	10.8	184.7	280.2	11.5	0.76
LSD(p=0.05)	0.2	NS	NS	43.7	39.6	1.8	0.12
Initial soil properties	-	284	9.6	142.2	141.5	10.3	0.72

Fig 42. On farm trial of enriched compost at KVKs of NE region.

Soil Quality under long term INM in *Sali* Paddy

The results of long term integrated nutrient management (INM) on rice for ten years demonstrated

the impact of enriched compost with reduction in chemical fertilizer even upto 75% of RD (NP) (Table 47). The treatment consisted of 25% RD of NP with 100% K along with enriched compost (2 t ha^{-1}) could sustain the rice production at 4.12 t ha^{-1} which was comparable with total inorganic fertilizer (3.98t ha^{-1}) (Table 47). The soil quality index (SQI) computed through principal component analysis (PCA) after ten years of INM in paddy showed the SQI ranges from 0.75 to 1.00. A low SQI (0.75) was observed in soil receiving no inputs.

Table 47. Rice yield and soil quality index under long term integrated nutrient management

Treatemnts	Rice Yield (t ha^{-1}) (mean of ten years)	Soil quality index	Sustainable Yield Index
Absolute Control	2.59 (±0.24)	0.75	0.76
100% RD of NPK(40:20:20)	3.98 (±0.35)	0.87	0.81
50% RD of NP + 100% K + Biofertilizers	3.95 (±0.22)	0.96	0.87
50% RD of NP + 100% K+ Enriched Compost@1t/ha	4.01 (±0.24)	0.98	0.87
25% RD of NP + 100% K+ Enriched Compost@2t/ha	4.12 (±0.25)	1.00	0.88
Biofertilizer + Compost@1t/ha	3.44 (±0.24)	0.94	0.81
LSD (p=0.05)	0.20	-	-

1.5. Demonstrations of improved bionutrient packages for pulses and other crops (RAU)

Rice is cultivated on 3.3 million ha area in Bihar with production of 7.5 million tones and the productivity of 2.28 t/ha (Table 48). The technology for direct seeded rice is gradually finding favor with the farmers as it saves irrigation water, minimizes labour costs and reduces the production costs by one-third. There is thus a need to develop suitable biofertilizer package for direct seeded rice. Field experiments were conducted for two years in an alluvial, calcareous soil (32% calcite) in rice-lentil rotation at RAU farm using Swarna sub-1 and Prabhat varieties (Table 48). *Azospirillum* and *Bacillus* were applied to seeds at sowing followed by surface application of cyanobacteria after receipt of first rainfall after seeding. The increase in grain yield due to microbial inoculants varied from 10-20% among the treatments.

Table 48. Effect of microbial inoculants on grain and straw yield of direct seeded rice (2014-16)

Treatment	2014		2015	
	Grain (Mg ha^{-1})	Straw (Mg ha^{-1})	Grain (Mg ha^{-1})	Straw (Mg ha^{-1})
Control	1.94	2.89	1.80	2.80
Microbial inoculants	2.28	3.46	2.03	3.46
50% NPK	2.80	3.85	2.48	4.07
50% NP + MI	3.16	4.39	2.82	4.32
100% N	3.53	4.72	3.23	4.76
100% N + MI	4.21	5.86	3.59	5.17
150% NPK	4.47	6.33	3.81	5.45
LSD (p = 0.05)	0.28	0.31	0.25	0.23

In a field experiment on lentil (var. Arun), two strains of *Rhizobium* (Rajendra *Rhizobium* Tal - RRT I, RRT II) and one strain of *Bacillus* sp. (Rajendra *Bacillus* Diara- RBD I) singly or in combination were tested (Table 49). Combined inoculation of RRTII + RBDI significantly increased the yields and BNF attributes value of grain and straw yield, no. of nodules /plant and ARA. The use of *Bacillus* as co-inoculant of *Rhizobium* was more effective than *Rhizobium* alone.

Table 49. Effect of *Rhizobium* and *Bacillus* coinoculation on yield of lentil

	Grain (Mg ha^{-1})	Straw (Mg ha^{-1})	Nodule no. $plant^{-1}$ (45 days)	ARA µM C_2H_4 g^{-1} nodule
Control	0.90	1.08	5.2	0.58
RRT I	0.95	1.14	7.0	0.86
RRT II	1.10	1.43	11.5	1.22
RRT I + RBD I	1.22	1.71	13.1	1.48
RRT II + RBD I	1.53	1.99	15.8	2.23
LSD (p = 0.05)	1.1	2.3	1.2	-

Bio-fertilizer technology demonstrations were done in rice and pulses crops in famer fields in Samastipur, Vaishali, Muzaffarpur, East Champaran, West Champaran, Katihar and Bhagalpur districts of Bihar (Table 50 and 51). The inoculants used were *Rhizobium* and *Bacillus* sp. for lentil and urdbean and cyanobacteria, *Azospirillum* and enriched mycostraw for rice. The technologies resulted in yield increases from 5 to 15% over farmer's practice. Varietal difference did not have any impact on the response of bio-fertilizer intervention. Imbalance and indiscriminate use of chemical fertilizers were more common among poor farmers due to fragmented land (Table 50 and 51). Increase in lentil yield was 13-24%; response was poor in diara lands of Katihar and Bhagalpur district on urdbean (4-8%) (Table 52). This showed that there is a greater need to screen for more efficient strains of rhizobia of pea group.

Table 50. Rice yield (q ha^{-1}) with bionutrient package in farmer fields in Bihar (2014-15)

	Resource poor farmers			Resource rich farmers		
Variety	FP	FP + BP	Fertilizer dose (kg ha^{-1})	FP	FP + BP	Fertilizer dose (kg ha^{-1})
Lal Dhan	11.6	13.4	Urea-130	-	-	(Tribal area)
Rajendra Bhagwati	19.3	22.0 (14.0)*	Urea-152	31.8	35.3 (11.0)	Urea – 40; DAP-60 MOP-40; $ZnSO_4$-15
Rajendra Masuri	15.2	17.3 (13.7)	Urea–75 DAP-60	29.2	31.5 (7.8)	Urea-80; DAP – 20 MOP – 20; VC – 2 t/ha
Rajendra Sweta	21.9	24.7 (12.3)	Urea–90 DAP-50	32.5	36.0 (10.7)	Urea-80; DAP – 30 Vermi compost (VC)– 4t/ha
PHB – 71	-	-		38.0	41.8 (10.0)	Urea-100; DAP – 40 MOP – 27; $ZnSO_4$ – 13
6444	-	-		40.3	43.1 (6.9)	Urea – 80; DAP – 60 MOP – 40; $ZnSO_4$ – 10

* % increase over control

Table 51. Rice yield (q ha^{-1}) with bionutrient package in farmer fields in Bihar (2015-16)

Variety	Resource poor farmers			Resource rich farmers		
	FP	FP + BP	Fertilizer dose (kg ha^{-1})	FP	FP + BP	Fertilizer dose (kg ha^{-1})
Parmal	16.1	18.4 (14%)	Urea-110Kg/ha DAP – 40 kg/ha	24.8	27.2 (9.2)	Urea-50; DAP-40 MOP-20
Rajendra Bhagwati	23.5	25.4 (8%)	Urea-130Kg/ha DAP-60kg/ha	33.6	35.9 (5.0)	Urea – 80;DAP-40 MOP-40; $ZnSO_4$-10
Rajendra Masuri	20.3	22.0 (8.3)	Urea–80Kg/ha DAP-55kg/ha	-	-	

Table 52. Biofertilizer technology impact on yield (q ha^{-1}) of lentil and urdbean in farmer fields (2014-15)

Lentil varieties	F1 (local)	F2 (local)	F3 (local)	F4 (Arun)	F5 (local)
Control	6.0	5.3	7.1	9.0	6.9
Rhizobium + *Bacillus* sp.	7.4	6.2	8.3	10.8	7.8
Urdbean (all traditional varieties)					
Control	15.1	18.4	9.0	16.6	8.3
Rhizobium + *Bacillus* sp.	16.0	19.2	10.9	17.7	10.4

4.6. Demonstrations on Biofertilizers in tribal areas (OUAT, JNKVV, KAU)

In three villages of Kalahandi district (Burat, Ghantmal and Chianpadar), 64 on-farm tribal area trials were conducted (Fig 43) with nine crops: namely tomato (15 no), cauliflower (13), cabbage (3), brinjal (4), cowpea (3), French bean (1), maize-cowpea (8), sole maize (8) and cotton (9); and during the summer, 22 crops were grown (Total 86). For vegetable crops on an average 400 m^2 area per farmer, for maize/ maize-cowpea 1000 m^2 per farmer and for cotton 2000 m^2/ farmer was the area grown and farmers were allowed to grow their desired crops for which they were supplied with critical inputs like seed, fertilizers, BFs and pesticides. The BFs were *Azotobacter, Azospirillum*, PSM and *Rhizobium*. The response to BFs inoculation ranged from 8.6- 22.0 %, lowest with greens (*saga)* and highest with cowpea. On an average bioinoculation of crop increased the economic yields by 14.6 % (Appendix 2).

One hundred and twenty four demonstrations were conducted during *Kharif, Rabi* and summer during 2015-16 by 45 tribal farmers in four villages in two tribal dominated districts Kalahandi and Rayagada in the state of Odisha. Out of 6600m^2 of demonstration area with twenty crops (at least 12 crops by each farmer) the tribal farmers were able to generate net benefit of Rs 42,855/- with the investment of Rs. 1050/- on bio inoculants, Rs 1000/- on vermicompost and Rs.50/- on lime as Paper mill Sludge (Total Rs.2100/-). The net return thus came to Rs.20/- earned per rupee investment. Bio-inoculation of crops improved the yields of vegetables crops (Appendix 2) by 10 to 40% over no inoculation. In green gram, the increase was 30%, maize by 25%, in fibre crop cotton and fruit crop banana by 17% and in oilseed crops from 10-15%.

The TSP Programme besides direct income generating programme also helped generating year round employment for neighbours.

- The programme could check migration of labourers out of the state.
- Creation of vermicompost pits out of the TSP fund helped preparation of good quality compost throughout the year and kept the rural environment clean and hygienic.
- Use of biofertilizers helped in saving at least 25 per cent of the cost incurred for the purchase of costly inorganic fertilizers.

Fig 43. Demonstrations on bioinoculation of crops in tribal areas of Odisha.

Research-Adoption-Impact continuum analysis of biofertilizer usage (JNKVV)

During 2014-15, 37 field demonstrations (Fig 44) were conducted on soybean (14), Maize (6), Wheat (5), Chickpea (6), Pea (4) and Lentil (2) on tribal farmers' fields in Mandla and Chhindwara districts. The average yield increase of different crops (Table 53) were: Soybean (21%); Maize (74%); Chickpea (18%): Wheat (6%); Pea (15%) and Lentil (12%) by using recommended dose of fertilizers along with biofertilizers over farmer's practice (FP) which consisted of imbalanced fertilization and no application of biofertilizers (Table 53). In case of oilseed and pulse crops *Rhizobium* and PSB biofertilizers were applied while with wheat *Azotobacter* and PSB biofertilizers were applied.

Table 53. Grain yield of field crops in tribal farmer demonstrations (kharif and rabi 2014-15) in Madhya Pradesh

Crop	Total No.					Average yield (kg/ha)	
	Variety	Demonstrations/ Farmers	Villages	Blocks	Districts	F.P.	R.D.F.+B.F.
Soybean	JS-9752	14	5	3	2	1424	1730 (21.5%)
Maize	JM-216	6	6	1	1	768	1343 (74.8%)
Chickpea	JG-16	6	4	4	2	768	1343 (74.8%)
Wheat	JW-273	5	3	2	2	1804	1916 (6.2%)
Pea	AP-3	4	4	3	2	692	797 (15.2%)
Lentil	Local	2	2	2	1	825	930 (12.7%)

*Figures in parenthesis are % increase over farmer's practice.

Fig 44. Demonstrations of biofertilizers in tribal areas of M.P. on chickpea and wheat.

During 2015 *kharif* field demonstrations were carried out on Soybean (20), Maize (2) and Paddy 1) in tribal farmers' fields in Mandla and Chhindwara districts. The average yield increase of different crops was: Soybean (12%); Maize (17%); Paddy (10%) due to application of recommended dose of fertilizers along with biofertilizers (*Rhizobium / Azotobacter* + PSB) over farmer's practice (FP) of imbalanced nutrition and no application of biofertilizers (Table 54). In rabi 2015-16, 20 demonstrations were laid on wheat, chickpea and pea crops in Jabalpur, Mandla, Dindori and Balaghat districts.

Table 54. Different field crops grain yield under field demonstrations (kharif 2015)

Crop	Variety	Total No.				Average	
		Demonstrations/ Farmers	Villages	Blocks	Districts	F.P.	R.D.F+B.F.
Soybean	JS-9560	20	11	6	4	628	703 (12%)
Maize	JM-216	2	1	1	1	435	510 (17%)
Paddy	MTU-1010	1	1	1	1	3380	3720 (10%)

*Figures in parenthesis are % increase over farmer's practice.

Popularization of biofertilizers in Wayand area (KAU, Thrissur)

Wayanad is located in the Western Ghats and tribals form about 17% of the total population of the district representing 36% of the total tribal population of the state. Tribal population includes Paniyas, Kurumas, Adiyars, Kurichyas, Ooralis, Kadans, and Kattunaikkans. Homestead farming is practiced in this district and the average size of holding is 0.68 ha. Biofertilizer preparations of *Azotobacter chroococcum, Azospirillum lipoferum* and PGPR mix-I (*Azotobacter chroococcum,* phosphate solubilizing *Bacillus megaterium* and a potash solubilizing *Pseudomonas* sp.) were recommended for seed treatment, seedling dip and soil application. Among spice crops, ginger and black pepper were selected for popularization of biofertilizers. Ginger is organically grown by the tribals, but no biofertilizers are being used. Pepper is grown largely along with coffee. Ginger and pepper cultivation in Wayanad has also substantially increased in recent times and the ginger produced is mainly marketed in the form of green ginger. Good quality biofertilizers were distributed to 125 beneficiaries in nine tribal settlements (Edakkal, Njamarath, Naduveetil, Nellara, Thannivayal, Nayakatty, Kuppadi and Vadachira (Table 55).

Table 55. Distribution of biofertilizers in the tribal settlements of Wayanad during 2015-16

Biofertilizer	Crop	No. of beneficiaries	Quantity supplied (kg)
Azotobacter chroococcum	Black pepper	70	500
	Bittergourd	50	100
Azospirillum lipoferum	Black pepper	70	300
	Ginger	55	110
	Bittergourd	50	100
PGPR Mix-I	Black pepper	70	300
	Ginger	55	200
	Bittergourd	40	40
	Cowpea	60	60

Three training programmes were organized in Naikatty, Nellara and Kolagappara where 97 farmers were educated on the types of biofertilizers and advantages of using biofertilizers (Fig 45). Demonstration of seed treatment was also held to familiarize them with the correct method of

application to ginger, black pepper and vegetables. Leaflets on biofertilizers were distributed among the farmers.

Fig 45. Training of tribal farmers on application of biofertilizers at Kolagappara and Naikatty for bittergourd and cowpea farmers.

Popularization of biofertilizers in Attapady, Palghat (KAU, Vellayani)

A consortium of biofertilizers (PGPR Mix I) developed by KAU was mass multiplied and distributed to 612 farmers of Attappady area engaged in the cultivation of vegetables, pulses, banana, sorghum, groundnut, ragi etc. One kg each of PGPR Mix I was distributed to each farmer (Fig 46). Tribal farmers, Agricultural Extension officers and other officials were trained on biofertilizer usage at Agali, Sholayoor and Puthoor Panchayat of hill tract. A total of 312 tribal farmers and 54 extension officers participated in the training programme. The method of application of the different biofertilizers was also demonstrated.

Fig 46. Distribution of biofertilizers and training at Agali and Puthoor.

In order to demonstrate the beneficial effect of the consortium of Biofertilizers (PGPR Mix I) field trials were laid out at two different locations of Attapady on Banana (Fig 47) with the objective of saving chemical fertilizers. A similar trial on arecanut was laid out at Puthoor panchayat which is under progress (Fig 48).

Fig 47. Banana field at Sholayoor before treatment with PGPR Mix I (Left) and after three months from treatment with PGPR Mix I (Right).

Fig 48. Trials on Arecanut Nursery at Puthoor.

4.7. Other Outreach Programmes

Demonstration of biofertilizers in upland rice (CRURRS)

Field demonstrations of biofertilizers in direct seeded upland rice and pigeon-pea under rainfed ecology were conducted during wet seasons of 2014 and 2015 in villages in Jharkhand state as detailed below.

2014 (Village Tilra, Block Mayurhanj, Dist. Chatra)

a. Efficacy of improved mass inoculum (MI) of native AMF developed through multiplying nucleus inoculum (NI) produced on improved substrate combination ('vermiculite: soil: FYM' fortified with Hoagland solution) was validated through on-farm trial in farmers' participatory mode. While traditional mass inoculum (developed from NI produced on substrate combination of soil: sand: FYM) @ 1.0 t/ha resulted 28.6% yield increase of upland rice (cv. CR Dhan 40), improved MI gave similar yield increase (+29.8%) at half the dose (0.5 t/ha).
b. Seed treatment with *Azotobacter* inoculum (source: BAU, Ranchi) resulted in 12.8% yield increase in direct seeded upland rice (cv. CR Dhan 40)
c. Seed treatment of *Rhizobium* inoculum (source: BAU, Ranchi) resulted in 43.2% yield increase in pigeon pea (cv. UPAS 120)

2015 (Joki *tola* of village Dasokhap, Block Churchu, Dist. Hazaribagh) – Tribal village

a. Improved mass inoculum of AMF @ 0.5 t/ha resulted in 56.8% yield increase in direct seeded upland rice (cv. Sahabhagidhan) over untreated control (0.55 t/ha) under severe moisture stress (drought).
b. Seed treatment with *Azotobacter* inoculum (source: BAU, Ranchi) resulted in 15.6% yield increase in direct seeded upland rice (cv. CR Dhan 40) under similar condition under severe moisture stress (drought).

Extension programmes (ANGRAU)

Training of different farming groups on the usage of liquid as well as solid biofertilizers was done regularly (Fig 49). Supply of mother cultures of BGA is done along with training to farmers for on-farm multiplication.

Fig 49. Farmers of Andhra Pradesh are being trained on the usage of liquid biofertilizers.

YSPUHF, Solan

Ten lectures given for Officers /Farmers in training programmes along with two radio talks on the use of PGPR/Biofertilizers in fruit crops/vegetable crops. Trials cum demonstrations on the use of PGPR on cauliflower and pea conducted at three locations of Solan and Kangra District and on the use of PGPR for improved growth and yield of apple at five different locations of district Shimla.

MAU, Parbhani

Three days training programme on 'Liquid Biofertilizer Production Technology" was organized by AINP centre, Parbhani for the technical staff working in the production units (30 no.) of biofertilizers of Maharashtra government. They were also imparted training on quality control. One book entitled "Liquid Biofertilizer Production Technology" authored by Syed Ismail and Vilas Patil was also released.

AAU, Jorhat

Two years experiment (2013-14 and 2014-15) conducted in collaboration with the Department of Horticulture, AAU on the use of microbial consortium *(Azospirillum, Azotobacter an*d PSB) and organic inputs (compost, vermicompost and enriched compost) on the yield and quality of cauliflower showed the superior performance of enriched compost (5.0 t ha^{-1}) with microbial consortium in increasing the curd yield (20.9 t ha^{-1}) (Table 56). However the recommended NPK (80:60:60 kg ha^{-1}) produced significantly highest curd yield (28.08 t ha^{-1}). On contrary, the quality

parameters of cauliflower curd like ash content (6.8%) and ascorbic acid (60.1 mg $100g^{-1}$) were significantly highest with enriched compost (5.0 t ha^{-1}) with microbial consortium (Table 56).

Table 56: Curd yield and quality parameters as influenced by organic inputs

Treatments	Yield (t ha^{-1})	Ash content (%)	Ascorbic acid (mg$100g^{-1}$)
RDF (N:P:K :80:60:60 kg ha^{-1})	28.1	6.3	37.2
Rock phosphate(RP) + Consortium	6.9	5.7	37.3
RP + Consortium + Compost (2.5 t ha^{-1})	12.9	6.1	43.3
RP + Consortium + Compost (5.0 t ha^{-1})	13.7	6.3	47.6
RP + Consortium + Vermicompost (2.5 t ha^{-1})	17.4	6.2	50.7
RP + Consortium + Vermicompost (5.0 t ha^{-1})	18.6	6.6	56.7
Enriched Compost (2.5 t ha^{-1}) + Consortium	19.2	6.3	60.0
Enriched Compost (5.0 t ha^{-1}) + Consortium	20.9	6.8	60.1
LSD (p=0.05)	2.5	0.03	3.1

In a DBT-NER Twinning Programme, three native isolates of *Bacillus thuringiensis* viz., $S5C_3$, $S25C_1$ and $J11C_5$ were found to be effective against two important polyphagous pests, *Spodoptera litura* and *Helicoverpa armigera.* The *Bt* isolates $J11C_5$ (LC_{50}=21ppm), G20W (LC_{50}=26 ppm) and $S5C_3$ (LC_{50}=29 ppm) showed potential for developing biopesticide formulation for *S. litura*.

In a collaborative programme on biological management of pests and diseases of tea ecosystem by native antagonists with Department of Plant Pathology, AAU, *Beauvaria bassiana, Paecilomyces lilacinus* and *Trichoderma spp.* were isolated from the soils of tea ecosystems. *Metarhizium anisopliae* was isolated from insect cadavers. *Trichoderma harzianum* was found effective against *Poria hypobrunnea* by 53%. *P. lilacinus* was effective in causing mortality (67%) to semilooper. *Metarhizium anisopliae* bioformulation was found effective in controlling termite up to one year after application.

IISS, Bhopal

In an institute outreach programme of IISS, Bhopal on "Integrated Assessment of Some IISS Technologies for Enhancing Agro-Ecosystem Productivity and Livelihood Sustainability" - (PI: Dr. Shinogi K.C.) biofertilizer technology for soybean and wheat was demonstrated in nine farmers fields of Mengra Kalan village, Berasia Tehsil, Bhopal District. Biofertilizers comprised of *Rhizobium* and Plant growth promoting bacteria consortium (mixture of three PGPR strains) for soybean; and PGPR only for wheat. The Biofertilizers were applied on the seeds as carrier based inoculants in two treatments of Integrated Plant Nutrient Supply System. In soybean these were i) Biofertilizers + 50%NPKS + 5t FYM/ha; ii) Biofertilizers + 50%NPKS + 2t/ha of Phospho-Sulpho-Nitro Compost. In wheat it was Biofertilizers + 75%NPKS application in both the above treatments. Soybean and wheat yield increased in INM mode with Biofertilizer by ~10-15% with FYM and 20-25% with enriched compost over farmers' practice.

In IISS training programmes-workshops, model training courses, summer and winters schools sponsored by ICAR, state governments, ATMA and other developmental agencies, Dr. D.L.N.Rao, PC (BF) delivered lectures on biofertilizers, soil health as well as two radio talks on

entrepreneurship for setting up R&D and production units of Biofertilizers. An awareness programme on importance of soil testing and biofertilizers was given to tribal farmers of Tamia Block, District Chhindwara, M.P., as well as on soil health, biofertilizers and importance of pulses in several programmes in villages around Bhopal in Mera Gaon Mera Gaurav Programme.

4.8. Biofertilizer production

JNKVV, Jabalpur

The details of production and sale of biofertilizer packets of *viz., Rhizobium, Azotobacter, Azospirillum,* phosphate solubilizing microorganisms, *Trichoderma* and BGA at JNKVV, Jabalpur is shown in Table 57.

Table 57. Biofertilizer Production and Supply (2014-16)

S.No.	Biofertilizer	No. of packets	Sale amount (Rs.)
1	*Rhizobium**	1,78,099	35,62,000
2	*Azotobacter**	37,178	7,43,560
3	*Azospirillium**	12,174	2,43,480
4	PSB**	1,89,829	47,45,725
5	*Trichoderma***	61,512	15,37,800
6	Soil Based BGA#	6,205	4,96,400
7	*Pseudomonas* @	142	28,400
	Total	4,85,139	113,57,365

*200 g/pkt @ Rs. 20/- ** 250 g/pkt @ Rs. 25/ #2 kg/pkt @ Rs. 80/- @ 1kg/pkt @ Rs. 200/-

CCS HAU, Hisar

The Biofertilizer unit of CCS HAU, Hisar is producing around 15 to 17.5 Kiloliters of liquid biofertilizer each year and thus generating revenue of Rs. 30-35 lakhs per year. About Rs. 15-25 lakhs of revenue is generated from the bio-inoculants developed in AINP on Soil Biodiversity and Biofertilizers and its predecessor AICRP on BNF (Table 58).

Table 58. Details of liquid biofertilizer production and sale of the cultures developed in BNF project, Hisar centre

Name of the organism	Crop	No. of 50 ml. vials sold	Sale cost (Rs.)
Year 2014-15			
Pseudomonas sp. (PSB)	All crops	1,83,577	18,35,770
Rhizobium /Bradyrhizobium/ Mesorhizobium	Berseem/ Mungbean/ Chickpea	1,03,395	10,33,950
Total		2,86,972	28,69,720
Year 2015-16			
Pseudomonas sp. (PSB)	All crops	85,264	8,52,640
Rhizobium /Bradyrhizobium/ Mesorhizobium	Berseem/ Mungbean/ Chickpea	16,627	1,66,270
Total		1,01,891	10,18,910
Grand Total		3,88,863	38,88,630

ANGRAU, Amaravathi

Using the strains of AINP on Soil Biodiversity and Biofertilizers the centre has produced 309.5 MTs of carrier based biofertilizers and 44.0 MTs of liquid biofertilizers during 2014-16 worth Rs. 255.8 Lakhs.

Table 59. Biofertilizers produced with the cultures of ICAR BNF Scheme at ARS, Amaravathi.

Year	Type of Biofertilizer produced (Metric Tons)		Total value (Rs. in Lakhs)
	Powder formulation	Liquid formulation	
2014-15	125.5	17.0	101.2
2015-16	184.0	27.0	154.6
Total	309.5	44.0	255.8

IARI, New Delhi

Table 60. Sale of Biofertilizers and revenue generated through sale of biofertilizers and pure cultures

Year	Bacterial biofertilizers (*Rhizobium, Azotobacter, Azospirillum*, PSB)	BGA	AM fungi	Compost inoculant	Revenue generated (in Rs.)
2014-15	7000	1000	1100	750	3,65,000
2015-16	7500	1200	1300	750	3,78,000

MKV, Parbhani

In order to increase the shelf life of biofertilizers, to avoid contamination and easy delivery the Biofertilizer unit, VN Marathwada Agricultural University, Parbhani is preparing liquid biofertilizers of *Rhizobium* (for all legumes grown in the region), *Azotobacter*, *Azospirillum* and PSB separately and their consortia (Rhizophos, Azotophos etc) in user friendly packaging sizes for an acre of seed treatment or application through micro-irrigation system. Awareness among the farmers was created about use of biofertilizers through direct discussion/ discussion on mobile, radio talk, farmers rallies, popular articles in local language, folders etc. Though the demand for liquid biofertilizers is increasing now a days particularly for the commercial crops like sugarcane, turmeric, cotton, citrus etc for their application through drip system but due to drought situation in Maharashtra state the sale dropped down from last two years (Fig 50).

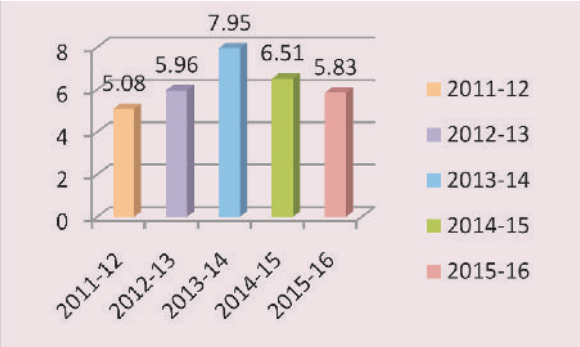


Fig 50. Biofertilizer sale over the years (Rs) from biofertilizer unit of VNMKV Parbhani.

Awards and Honours

1. Dr. D.L.N.Rao, PC (BF) worked as Member, Research Advisory Committee, National Bureau of Agriculturally Important Microorganisms, Mau, U.P.
2. Dr. D.L.N.Rao, PC (BF), 7th Professor S.K. Mukherjee memorial lecture award, IISS, Kolkata, 2015.
3. Dr. D.L.N.Rao, PC (BF) awarded a DBT-BBSRC project- Indo-UK Nitrogen Fixation Centre (IUNFC), under the Newton- Bhabha Fund as PI and Consortium Leader at IISS, Bhopal + 6 centres in India with University of Oxford+ 2 centres in UK (2016-19).
4. Dr. A.K.Saxena, IARI received Distinguished Scientist Award from Asian PGPR Society (2014).
5. Dr. A.K.Saxena, IARI inducted as a Fellow of Academy of Microbiologists of India (2014).
6. Dr. A.K.Saxena, IARI received the Prof G. Rangasamy Award (2015) of Association of Microbiologists of India.
7. Dr. A.K.Saxena, IARI joined as Director, National Bureau of Agriculturally Important Microorganisms, Mau, U.P.
8. Dr. N. Trimurtulu, ANGRAU received Best Scientist award from M/s. V. R. Durgaamba Charitable Trust, Chennai for his contributions in "Organic farming in Sugarcane crop" during 45th annual convocation of ANGRAU held at BV Nath Auditorium, Agricultural College, Bapatla.
9. Dr. Rajesh Gera, CCS HAU received "Best Paper Award" on the paper entitled "Exploring the potential of phosphate solubilising diazotrophic *Pseudomonas* sp Db76 as plant growth promoter for *Bt* cotton" published in Journal of Cotton Research and Development during 2014. The Award was given by Cotton Research and Development Association (CRDA) during AICCIP workshop held at TNAU, Coimbatore on 10th April, 2015.
10. Dr. D. Girija, KAU received the Indira Gandhi Gold Medal instituted by the Global Economic Progress and Research Association (GEPRA), Thiruvannamalai, Tamil Nadu on 19-11-2015.
11. Dr. Syed Ismail, MKV received ICAR sponsored Best Teacher's Award (2015) of Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra.
12. Dr. Syed Ismail and Dr V.D., Patil, MKV were awarded Radhakishan Shanti Malhotra Award (2015) for outstanding research work on "Identification of beneficial soil nutrient mobilizing cultures, their large scale production and supply to farmers in the form of liquid biofertilizers" by Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani, Maharashtra.
13. Dr. Syed Ismail, MKV received Best Paper Presentation Award at 1st International Conference on Agriculture & Horticulture Sciences (ICAHS-2015, ICAR-IARI) New Delhi, India during 6-7 June 2015 for a paper on 'Solubilization of insoluble zinc compounds by different microbial isolates in vitro condition'.
14. Dr. Rajesh Kaushal, YSPUHF received best poster award at the 2nd International conference on "Bio-resource and stress management" at PJTSAU, Rajendranagar, Hyderabad, Jan 2015.
15. Dr. Rajesh Kaushal, YSPUHF received best poster award in National symposium on "Modern agro-technologies for nutritional security and health" at Dr YSPUHF, Solan, April 2015.

Important Publications

Ansari, PG., Rao, DLN., Pal, KK. (2014) Diversity and Phylogeny of Soybean Rhizobia in Central India. *Annals of Microbiology* 64: 1553-1565.

Aparna, K., Pasha, MA. Rao, DLN. and Krishnaraj, PU. (2014) Organic Amendments as Ecosystem Engineers: Microbial, Biochemical and Genomic Evidence of Soil Health Improvement in a Tropical Arid Zone Field Site. *Ecological Engineering* 71: 268-277.

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Aparna, K.., Rao, DLN. and Balachandar D. (2016) Microbial Populations, Activity and Gene Abundance in Tropical Vertisols under Intensive Chemical Farming. *Pedosphere* 26: 725-732.

Balachandar, D., Chinnadurai, C., Tamilselvi, SM., Gopalaswamy, G., Arulmozhiselvan, K., Ilamurugu, K. and Rao, DLN (2014) Sustainable soil fertility: Insights from soil biochemistry and soil metagenomics of 100-years old permanent manurial trial. AINP on Soil Biodiversity - Biofertilizers Bulletin, Tamil Nadu Agric Univ, Coimbatore, p 4.

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अनुसंधान के प्रमुख अंश

जैव उर्वरकों के लिए सूक्ष्म जैव विविधता

- संपूर्ण भारतवर्ष से 20 प्रमुख दलहनीय फसलों से 2000 से अधिक राइजोबियम जीवाणुओं के उपादानों का पृथक्ककरण किया गया जिनमें से लगभग 700 उपभेद राजस्थान एवं हरियाणा के अर्धशुष्क एवं शुष्क क्षेत्रों, झारखण्ड के अम्लीय भूमि, बिहार एवं उत्तराखंड के 'ताल' भूमि से निकाले गए इनमें से 300 से अधिक उपभेदों का 16S rRNA जीन हेतु अनुक्रम किया गया।
- सोयाबीन आधारित फसल चक्रों में सोयाबीन फसल के साथ राइजोबियम जीवाणुओं की संख्या में वर्षा ऋतु के बाद लगभग 13 गुना वृद्धि हुई जबकि अनाज आधारित फसलों में यह वृद्धि केवल 1.7 गुना ही रही। कृषि प्रक्षेत्रों से बनी कार्बनिक खाद के नियमित उपयोग करने से मृदा में सोयाबीन राइजोबियम की संख्या में रासायनिक उर्वरकों वाले कार्यस्थल की तुलना में 1.5 गुना एवं जहाँ किसी भी प्रकार के उर्वरक/खाद का उपयोग नहीं किया गया उसकी तुलना में 2.5 गुना वृद्धि आंकी गई। धीमी गति से वृद्धि करने वाले (ब्रेडीराइजोबियम जेपोनिकम की अनुवांशिक समकक्ष) उपभेदों ने तेजी से वृद्धि करने वाले [राइजोबियम (एग्रोबैक्टिरियम) रेडियोबेक्टर के अनुवांशिक समकक्ष] अधिक नत्रजन स्थिरीकरण (+15%) किया। धीमी गति से वृद्धि करने वालों का अनुपात अनाज आधारित फसल चक्रों की तुलना से (64%) सोयाबीन आधारित फसल चक्रों में कम (38%) था।
- शुष्क क्षेत्रों की मृदाओं की राइजोबिया का तनाव सहनशक्ति एवं पी.जी.पी.आर. विशेषता के लिए प्रमाणीकरण किया गया। उच्च ताप एवं सूखा प्रतिरोधक उपभेदों को इनकी क्षमताओं के लिए आंका गया। अवर्षा वाली परिस्थितियों में टीकाकरण से क्लस्टर बीन, मूँगबीन एवं अरहर की पैदावारों में महत्वपूर्ण वृद्धि हुई। शुष्क मृदाओं के राइजोबियम में अनुवांशिक विविधता अधिक पाई गई।
- बिहार की नीची भूमि वाली धान 'देसारिया' की जड़ों के ऊतक में पाए जाने वाले राइजोबिया की बहुत अधिक अनुवांशिक समकक्षता जलीय दलहन 'एस्काइनोमीन' के तने एवं जड़ों की गठानों में पाए जाने वाले राइजोबिया से पाई गई। मेटाजीनोमिक विश्लेषण से पाया गया कि देसारइया धान की जड़े जीवाणुवीय प्रजातियों का बहुत बड़ा भंडार (2000 हजार से अधिक जीनोटाइप्स) है जिनमें 'आरकिया' सहित 29 फाइला है। मीथोनोजेन्स की तुलनात्मक प्रचुरता 'मिथाइलोट्राप्स' की तुलना में आधी थी।
- अम्लीय मृदाओं के अरहर, चना एवं सोयाबीन से प्राप्त कई राइजोबियम उपभेदों का प्रोटिओमिक विश्लेषण करने पर ज्ञात हुआ कि अम्लीय मृदाओं के प्रतिरोध उपभेदों में विशेष प्रोटीन अंतर है।
- उत्तर पर्वतीय क्षेत्रों के फसलों के जड़ीय क्षेत्र से पोटाश घोलक जीवाणुओं की 16S rRNA अनुक्रम करने पर उनकी पहचान बेसिलस सीरियस, क्लेबसिइएला वेरिकोला एवं क्लेबसिइएला प्रजाति के रूप में हुई।
- विभिन्न स्थानों पर खेतों पर किए गए परीक्षणों से ज्ञात हुआ कि जो फ्लोरोसेंट स्यूडोमोनास डी.ए.पी.जी. की उत्पत्ति करते हैं उनके द्वारा कालर एवं तने की गलने वाली बीमारी का कम आक्रमण हुआ जिससे मूंगफली की फली की पैदावार में सुधार हुआ।
- वी.ए. माइकोराइजा के नाभिकीय टीके को पोषक तत्वों से मजबूत करने पर धान की फसल में जैव उर्वरक की आधी मात्रा की आवश्यकता पड़ती है।
- मध्यप्रदेश की वर्टिसाल मृदाओं में एक्टिनोमाइसिटिस के A10 एवं A17 उपभेदों का पी.जी.पी.आर. (P3, P10, P25) का निरूपण करने से गेहूँ, सोयाबीन एवं चने (राइजोबियम के साथ) की पैदावार में महत्वपूर्ण

वृद्धि हुई।

- मध्यप्रदेश की वर्टिसाल मृदाओं में आर्थोबेक्टर उपभेदों का प्रयोग करने से सोयाबीन, धान, मक्का, चना एवं गेहूँ की पैदावार में महत्वपूर्ण वृद्धि प्राप्त हुई।
- पी.जी.पी.आर. (बेसिलस प्रजाति) एवं ए.एम.एफ. के एकत्रित उपयोग से मीठी चेरी के रोपे में वृद्धि हुई। हिमाचल प्रदेश में टमाटर एवं शिमला मिर्च पर पी.जी.पी.आर के उपयोग से इनकी पैदावार में महत्वपूर्ण वृद्धि प्राप्त हुई।
- हिमाचाल प्रदेश के पांच स्थानों पर बेसिलस लाइकिनिफार्मिस के तरल टीके का सेव की जड़ों में डालने पर पैदावार में प्रभावशाली वृद्धि आंकी गई।
- केजूरायिना इक्वीसेटिफोलिआ एवं एलनस निटिडा की जड़ीय गठानों से फ्रेंकिया का अलगाव कर निरूपण किया गया।
- उड़ीसा की अल्फीसॉल मृदाओं में जैव उर्वरकों का समन्वित पोषक तत्व प्रणाली के अंतर्गत कार्बनिक खादों एवं रासायनिक उर्वरकों के साथ प्रयोग करने से विभिन्न फसलों में उत्तम प्रदर्शन प्राप्त हुआ। मूँग के बीज को गोंद या साबूदाने के घोल को अंकितक की तरह अम्लीय मृदाओं में उपयोग करने से फसलों की पैदावार में वृद्धि हुई।
- एजोटोबेक्टर, एजोस्पिरिलम एवं पी.एस.बी. के संघटित उपयोग से जूट की फसल नत्रजनीय एवं स्फुरीय रासायनिक उर्वरकों की 50 प्रतिशत मात्रा कम लगी।

मृदा स्वास्थ्य मूल्याँकन के लिए मृदा जीनोमिक्स

- मेटाजीनोमिक विश्लेषण से ज्ञात हुआ कि जैविक कृषि वाली मृदाओं में केपिओट्रॉपिक जीवाणुओं की अधिकता पाई गई जो इस तथ्य का द्योतक है कि उन मृदाओं में पोषक तत्व की उपलब्धता अच्छी है। इसके अलावा प्रधान सिद्धांत वाले लाभकारी जीवाणुओं जैसे बेसिलस, स्ट्रेप्टोमाइसिज, स्यूडोमोनास, आर्थोबेक्टर एवं ब्रेडीराईजोबियम की भी तुलनात्मक आधिक्य था। श्वसन, प्रतिलिपि एवं रूपांतरण जैसी जैविक क्रियाऐं भी जैविक मृदाओं में अधिक पाई गई।
- समन्वित पोषक तत्व वाली प्रणाली के अंतर्गत एल्फीसॉल मृदाओं में एक दीर्घकालीन प्रयोग में यह पाया गया कि 16S rRNA जीन की प्रतिलिपियाँ नियंत्रण, रासायनिक या जैविक पद्धति से पोषित मृदाओं की तुलना में अधिक थी एवं रूबिस्को जीन cbbl form I फोटोसिंथेटिक एवं कीमोऑटोट्रापिक कार्बन स्थिरीकरण जीन में कोई अंतर नहीं था। यद्यपि काइटिनेज जीन की अधिकता कार्बनिक संशोधित मृदाओं (कार्बनिक खाद और समन्वित पोषक तत्व प्रबंधन) में रासायनिक परिपोषित मृदाओं या बिना परिपोषित मृदाओं की तुलना में अधिक थी।
- पोन्टीबेक्टर उमरिएनसिस प्रजाति नोव., ल्यूटिमोनस टॉलरेन्स प्रजाति नोव. एवं टेसारोकोक्कस फ्लेवस प्रजाति नोव. के अनूठे उपभेद हेक्सा क्लोरो साइक्लोहेक्सेन ढेर स्थल से खोजकर उनकी विशेषताऐं ज्ञात की।

जैव उर्वरक तकनीकि में सुधार

- जब कृषक बुआई के समय जैव उर्वरकों का उपयोग करने से चूक जाते हैं उस परिस्थिति में बुआई के पश्चात् उड़द में तरल जैव उर्वरकों का उपयोग पैदावार की कमीं में पूर्ति करते हैं। सूखे की परिस्थिति में भी तरल जैव उर्वरकों के उपयोग से अरहर की जड़ों की ग्रंथियों में बढ़वार एवं सकारात्मक पौध वृद्धि देखने को मिली।

- आंध्रप्रदेश में पोटाश घोलक जीवाणुओं के द्वारा चारे की पैदावार में वृद्धि पाई गई तथा 25 प्रतिशत पोटाश उर्वरक की बचत हुई।
- आंध्रप्रदेश में विभिन्न जीवाणुओं के मिश्रित एवं समन्वित उत्पाद द्वारा कृषि कुड़ा करकट का दो माह के अंदर सड़ाना संभव हुआ।

जैव उर्वरकों के उपयोग में विविधता एवं विस्तार

- जस्ता संगठित सूक्ष्मजीव उपभेदों जैसे ट्राईकोडर्मा विरिडी एवं स्यूडोमोनास स्ट्रयेटा ने महाराष्ट्र में सोयाबीन, मूँगफली एवं कपास की पैदावार बढ़ाने तथा भूमि में जस्ते की उपलब्धता तथा पौधों द्वारा उद्ग्रहण करने में अच्छा प्रदर्शन किया।
- उत्तर–पूर्व भारत में सूक्ष्म जीवों से समृद्ध कंपोस्ट के उपयोग से तीखी मिर्च की पैदावार में वृद्धि हुई। असम के कृषि विज्ञान केन्द्रों में जैविक पैकेज के अंतर्गत समृद्ध कंपोस्ट एवं एजोला के सकारात्मक उपयोग पर प्रदर्शन किए गए।
- बिहार का 'ताल' भूमि में सीधी बुआई वाली धान पर एजोस्पिरिलम एवं बेसिलस का बीज निषेचन एवं पौध रोपण के पश्चात् नील हरित काई के उपयोग से पैदावार में महत्वपूर्ण वृद्धि प्राप्त हुई। राइजोबियम एवं बेसिलस के सम्मिलित उपयोग से मसूर एवं उड़द की पैदावार में सुधार हुआ। जैव पोषक तत्वों आधारित पैकेज के अंतर्गत नील हरित काई, एजोस्पिरिलम एवं स्यूडोमोनास सवंर्धित 'माइकोस्ट्रा' से धान की पैदावार में उल्लेखनीय वृद्धि हुई।
- मध्यप्रदेश में किसानों के खेतों में 60 प्रदर्शन (मंडला, छिंदवाड़ा, जबलपुर, डिण्डौरी, एवं बालाघाट जिलों में) किए गए जिसमें अनुशंसित रासायनिक उर्वरकों की मात्रा के साथ जैव उर्वरकों के उपयोग से सोयाबीन, मक्का, गेहूँ, चना, मटर, मसूर एवं धान की पैदावार में किसानों द्वारा व्यवहारित (बिना जैव उर्वरकों के असंतुलित रासायनिक उर्वरक) पद्धति की तुलना में उल्लेखनीय वृद्धि का प्रदर्शन किया गया।
- उड़ीसा राज्य के कलाहांडी एवं रायगड़ा जिलों में अनुसूचित जन जाति वाले किसानों के खेतों में जैव उर्वरकों के उपयोग पर 210 प्रदर्शन सब्जियों, दालें, अनाज, तिलहन एवं रेशे वाली फसलों पर डाले गए एवं यह पाया गया कि कंपोस्ट, जैव उर्वरक एवं चूने के ऊपर प्रति एक रूपया खर्च पर 20 रू. का महत्वपूर्ण आर्थिक लाभ हुआ। अनुसूचित जनजाति कार्यक्रम के अंतर्गत किसानों को अतिरिक्त आय के अलावा पूरे वर्ष रोजगार प्राप्त हुआ जिससे लोगों का राज्य के बाहर प्रवसन रोकने में सहायता मिली। वर्मीकंपोस्ट टेंक बनाने से किसानों को पूरे वर्ष भर उत्तम गुणवत्ता वाला कंपोस्ट मिला एवं इसके अतिरिक्त ग्रामीण वातावरण को साफ एवं स्वस्थकर रखने में सहायकता मिली। इसके साथ–साथ महंगे रासायनिक उर्वरकों के उपयोग पर लगभग 25 प्रतिशत बचत हुई।
- केरल राज्य के वायनाड जिले में 10 संस्थनों में अनुसूचित जनजाति के 125 किसानों को अदरक, काली मिर्च एवं सब्जियों के उत्पादन हेतु एजोस्पिरिलम लाइपोफेरम, एजोटोबेक्टर क्रूकोक्कम एवं पी.जी.पी.आर. के संयुक्त जैव उर्वरक प्रदाय किया गया। केरल के ही अट्टापेडी एवं पालघाट में पी.जी.पी.आर. मिक्स 1 उर्वरक 600 किसानों को वितरित किए गए जो सब्जियों, दालें, केला, ज्वार, मूँगफली एवं रागी आदि के उत्पादन में संलग्न थे। 400 किसानों से अधिक एवं 50 विस्तार अधिकारियों को दोनों स्थानों पर जैव उर्वरकों के महत्व एवं उपयोगिता पर प्रशिक्षण भी दिए गए।

- आंध्रप्रदेश के किसानों में तरल जैव उर्वरक लोकप्रिय हुआ है तथा उनके द्वारा यह बताया गया कि विभिन्न फसलों में 10–15 प्रतिशत अतिरिक्त पैदावार के साथ 20–25 प्रतिशत रासायनिक उर्वरकों के उपयोग में कमीं आई। महाराष्ट्र में तरल जैव उर्वरक की मांग उन किसानों में बढ गयी है जो कपास, हल्दी, गन्ना, संतरा और अनार की पैदावार टपकन सिंचायी की सहायता सें कर रहे है।
- मृदा जैव विविधता एवं जैव उर्वरकों पर अखिल भारतीय नेटवर्क परियोजना के अंतर्गत विभिन्न सूक्ष्मजीव उपभेदों का उपयोग कर ANGRAU, JNKVV एवं MAU कृषि विश्वविद्यालयों ने जैव उर्वरकों का व्यापारिक उत्पादन कर लगभग 381.7 लाख रू. अर्जित किए जो कि परियोजना पर 2014–16 में आई लागत का लगभग 77 प्रतिशत हिस्सा है।

Appendix I: MALDI-TOF-TOF data of protein spots and their attributed gene functions (pigeonpea)

S.no.	Protein Identification (MALDI-TOF-TOF)	Attributed Functions	Accession No.
1	Chemotaxis Protein CheR [*Myxococcus xanthus* DK]	Involved in modulation of the chemo-taxis system	gi|108758057
2	TTT family tricarboxylate transporter, receptor protein, [*Achromobacter piechaudii* ATCC 43553]	Constituents of primary and secondary active transport systems	gi|293603568
3	Extra-cytoplasmic solute receptor family protein 18 [*Achromobacter xylosoxidans* A8]	Involved in solute transport, involved for the recruitment of the solute and its presentation to the membrane complex	gi|311104125
4	Exported protein [*Achromobacter xylosoxidans* C54]	Required for virulence	gi|422322729
5	Aldo / keto reductase [*Rhizobium etli* Brasil 5]	Super-family of enzymes that catalyze redox transformations, involved in biosynthesis, intermediary metabolism, and detoxification	gi|218510830
6	Valyl-tRNA synthetase [*Microscilla marina* ATCC 23134]	Cellular metabolism	gi|124009039
7	Deoxyribodipyrimidine photolyase [*Mariniradius saccharolyticus* AK6]	Cellular metabolism	gi|440749714
8	Molybdate ABC super-family ATP binding cassette transporter, binding protein [*Achromobacter piechaudii* ATCC 43553]	Uses hydrolysis of ATP to energize diverse biological systems, involved in the export or import of a wide variety of substrates ranging from small ions to macromolecules, major function is to provide essential nutrients to bacteria.	gi|293606431
9	Branched-chain amino acid ABC super-family ATP binding cassette transporter, amino acid-binding protein [*Achromobacter piechaudii* ATCC 43553]	Provide movement of diverse solutes, facilitates net uptake of solutes into bacterial cells	gi|293602433
10	Exported protein [*Achromobacter xylosoxidans* C54]	Required for virulence	gi|422323639
11	Extra-cytoplasmic solute receptor family protein 4 *[Achromobacter piechaudii HLE]*	Involved in citrate uptake, functions as receptors for osmotic solutes produced and accumulated by bacterial cells under stress (salt)	gi|421483536
12	TTT family tricarboxylate transporter, receptor protein [*Achromobacter piechaudii* ATCC 43553]	Constituents of primary and secondary active transport systems	gi|293602883
13	ABC super-family ATP binding cassette transporter, binding protein [*Achromobacter piechaudii* ATCC 43553]	Provide movement of diverse solutes, facilitates net uptake of solutes into bacterial cells	gi|293603959
14	Receptor family ligand binding region family protein 10 [*Achromobacter piechaudii* HLE]	Cellular metabolism Catalysis of the transfer of a methyl	gi|421482768

S.no.	Protein Identification (MALDI-TOF-TOF)	Attributed Functions	Accession No.
15	Chemotaxis protein CheR [*Myxococcus xanthus* DK 1622]	Catalysis of the transfer of a methyl group from S-adenosyl-L-methionine to a substrate	gi|108758057
16	Tat pathway signal sequence domain-containing protein 9 [*Achromobacter piechaudii* HLE]	Operates in plant thylakoid membranes and the plasma membranes of most free-living bacteria. In bacteria, it is responsible for the export of a number of proteins to the periplasm, outer membrane or growth medium, can transport large folded proteins (even oligomeric proteins) across the tightly sealed plasma membrane	gi|421483968
17	TTT family tricarboxylate transporter, receptor protein [*Achromobacter piechaudii* ATCC 43553]	Constituents of primary and secondary active transport systems	gi|293604353
18	Secreted protein [*Achromobacter xylosoxidans* C54]	Gram-negative bacteria secrete a wide range of proteins, functions include biogenesis of organelles (pilli and flagella), nutrient acquisition, virulence, and efflux of drugs and other toxins. Six distinct secretion systems have been shown to mediate protein export through the inner and outer membranes of Gram-negative bacteria.	gi|422322292
19	TRAP-T family tripartite ATP-independent periplasmic transporter, binding protein [*Achromobacter piechaudii* ATCC 43553]	Ubiquitous in prokaryotes, but absent from eukaryotes, are high-affinity, Na(+)-dependent unidirectional secondary transporters	gi|293606006
20	3-Hydroxyisobutyrate dehydrogena se MmsB [*Janthinobacterium* sp. HH01]	Cellular metabolism	gi|445497174
21	Pyruvate Kinase [*Xylella fastidiosa* Temecula1]	Cellular metabolism	gi|28199715
22	Aspartyl-tRNA synthetase [*Rhodopirellula baltica* SH28]	Cellular metabolism	gi|421610931
23	UvrB/UvrC protein [*Thermaerobacter marianensis* DSM 12885]	Cellular metabolism	gi|317123128

MALDI-TOF-TOF data of protein spots and their attributed gene functions (chickpea)

S.no.	Protein Identification (MALDI-TOF-TOF)	Attributed Functions	Access ion No.
1	Mannitol dehydrogenase [*Stenotrophomonas maltophilia* JV3]	Involved in host-pathogen interactions, pathogens and host plants appear to recruit existing enzymes or metabolites to serve unique functions during host–pathogen interactions.	gi|3442088 34
2	Branched-chain amino acid ABC superfamily ATP binding cassette transporter, binding protein [*Achromobacter piechaudii* ATCC 43553]	Involved in the import of essential nutrients and the export of toxic molecules, but they can also mediate the transport of many other physiological substrates, translocate a wide variety of substrates across extra - and intracellular membranes, including metabolic products.	gi|293605606

S.no.	Protein Identification (MALDI -**TOF-TOF)**	**Attributed Functions**	**Access ion No.**
3	OmpW family protein [*Stenotrophomonas maltophilia* AU12-09]	Family of evolutionarily r elated proteins from the outer bacterial membrane, includes outer membrane protein W (OmpW) proteins from a variety of bacterial species, involved in receptor functions, widespread in Gram -negative bacteria, may be involved in the protection of bacteria ag ainst various forms of environmental stress, could be involved in the transport of small hydrophobic molecules across the bacterial outer membrane.	gi|460867004
4	Outer membrane protein W precursor [*Stenotrophomonas maltophilia* D457]	Involved in protein i mport (mitochondrial protein import).	gi|386719763
5	Elongation factor Tu, partial [*Pseudomonas geniculata* N1]	Involved in protein synthesis, part of the mechanism that synthesizes new proteins by translation at ribosomes.	gi|408821473
6	Extra-cytoplasm ic solute receptor family protein 166 [*Achromobacter arsenitoxydans* SY8]	Involved in solute transport processes, super -family members exhibit the unusual capacity to function in conjunction with auxiliary proteins that modify transport process by providing (i) high -affinity solute reception, (ii) altered energy coupling, and (iii) additional yet to be defined functions.	gi|359800951
7	Electron transfer flavoprotein alpha subunit [*Stenotrophomonas maltophilia*]	Involved in cellular functions, functions as a specific electron acceptor for primary dehydrogenases, involved in transferring the electrons to terminal respiratory systems, such as electron -transferring -flavoprotein dehydrogenase.	gi|63054922

Appendix II: Improved yields with biofertilizers under TSP in Kalahandi district (2014-15)

Sl. No.	Crop	Area (m^2)	Season	No.of demos.	Average yield (kg)		BFs response (%)	BFs used
					Without BF	With		
1.	Vegetables							
	Tomato	400	Rabi	15	1018	1160	14.0	**
	Cabbage	400	Rabi	3	1132	1302	15.0	**
	Cauliflower	400	Rabi	13	728	823	13.1	**
	Bean	400	Rabi	1	230	278	21.0	*
	Onion	400	Rabi	6	578	662	14.5	**
	Brinjal	400	Summer	4	540	608	12.5	**
	Capsicum	400	Rabi	4	333	380	13.4	**
	Cowpea	400	Kharif	3	1423	1736	22.0	*
	Chilli	400	Summer	2	106	117	10.6	**
	Okra	400	Summer	4	275	315	14.5	**
2	Pulses							
	Arhar	400	Kharif	2	60	70	16.7	*
3.	Cereals							
	Maize-cowpea	1000	Kharif	8	2534	2772	9.4	**
	Maize	1000	Kharif	8	2624	2852	9.2	**
4	Fibre crops							
	Cotton	2000	Kharif	9	421	483	14.7	**
5	Fruit crops							
	Banana	1000	Year round	2	7334	8705	18.7	**
6	Greens							
	Leafy veg.	50	Summer	2	70	76	8.6	**
	Total area	9450		86				

**Rhizobium* seed inoculation + PSB @ 6 kg ha^{-1} ***Azotobacter, Azospirillum* and PSM

Extra income due to bioinoculation of crops (2014-15)

Sl.No.	Crop	Sale price (Rs.)	Gross Income (Rs.)	Cost of BFs (Rs)	Net income (Rs)
1	Tomato	12	1704	50	1654
2	Cabbage	8	1360	50	1310
3	Cauliflower	20	1900	50	1850
4	Bean	18	864	50	814
5	Onion	25	2100	50	2050
6	Brinjal	22	1496	50	1446
7	Capsicum	35	1645	50	1595
8	Cowpea	12	3756	50	3706
9	Chilli	60	660	50	610
10	Okra	18	720	50	670
11	Arhar	40	400	25	375
12	Maize	5	1190	50	1140
13	Cotton	40	2480	200	2280
14	Banana (1000 m^2)	13	18408	200	18208
15	Greens (Leafy)	20	120	20	100
	Total area (9450 m^2)	-	38803	995	37808

Bioinoculation of crops over an area of 9450 m^2 came to Rs.2180 (BFs -995 + Vermicompost Rs.1140 + lime Rs.45). The net income was Rs.37808. Therefore per rupee invested on bioinoculation of crops earned Rs.17.35.

Improved yields with biofertilizers under TSP in Kalahandi and Rayagada districts of Odisha (2015-16)

Sl.No	Crop	Season	No. of Demonstrations	Av. yield(kg/200m^2)		BF Response (%)	BFs used
				Without BF	With BF		
A–Vegetables							
1	Brinjal	*Kharif*	05	408	458	12.2	**
2	Tomato	*Rabi*	11	1188	1378	16.0	**
3	Cauliflower	*Rabi*	12	715	800	12.0	**
4	Cabbage	*Rabi*	07	1232	1410	14.5	**
5	Broccoli	*Rabi*	03	233	255	9.5	**
6	Capsicum	*Rabi*	03	310	350	13.0	**
7	Bitter Gourd	*Summer*	02	178	202	13.5	**
8	Pointed Gourd	*Summer*	01	220	265	20.4	**
9	Cowpea	*Kharif*	03	1560	1900	21.7	*
10	Colocasia	*Rabi-Summer*	02	370	426	15.1	**
11	Watermelon	*Rabi-Summer*	03	2180	3080	41	***
12	Onion	*Rabi*	14	560	638	13.9	**
13	Chilli	*Kharif-Summer*	06	94	104	10.6	**
B-Cereals							
1	Maize	*Kharif*	03	2688	3400	26.5	**
C- Pulses							
1	Greengram	*Rabi*	07	10.8	14.0	29.6	*
2	Lentil	*Rabi*	01		-		*
D-Oilseeds							
1	Soybean $^{\Delta}$	*Kharif*	07	11.6	13.4	15.5	*
2	Sunflower	*Rabi*	03	36.0	39.9	10.8	**
3	Sesamum	*Summer*	01	18	20.7	15.0	**
E- Fruits							
1	Banana(1000m^2)	Round the Year	04	7500	8250	17.5	**
F-Fibre crops							
1	Cotton(2000m^2)	*Kharif-Rabi*	26	440	514	16.8	**
Total			**124**				

*Seed inoculation with *Rhizobium* + Soil applied with PSB @ 6 kg ha^{-1}.
** *Azotobactor* + *Azospirillium* + PSM (1:1:1), 4kg each ha^{-1} inoculated to prelimed vermicompost
*** *Azotobactor* + *Azospirillium* + PSM+ A.M (as above).
Δ- Crop suffered severe drought

Note: - Biofertilizers as inoculated (1:25) and incubated (5%) limed vermicompost were used twice during crop growth period: first at the time of sowing / planting of seedlings and second at top dressing except for Green gram, Bitter gourd, *Sesamum*.

Extra income due to bioinoculation of crops (2015-16)

Sl. No	Crop	Sale Price (Rs/Kg)	Gross extra income (Rs.) due to BF use/200m^2	Cost of BFs (Rs.) used)	Net Income (Rs./200m^2)
A – Vegetables					
1	Brinjal	20	1350	50	1300
2	Tomato	15	2850	50	2800
3	Cauliflower	20	1700	50	1650
4	Cabbage	10	1780	50	1730
5	Broccoli	25	850	50	800
6	Capsicum	20	1200	50	1150
7	Bitter Gourd	15	360	25	335
8	Pointed Gourd	50	2250	50	2200
9	Cowpea	15	5100	25	5075
10	Colocasia	35	2000	50	1950
11	Watermelon	5	4500	50	4450
12	Onion	24	1880	50	1830
13	Chilli (green)	50	550	50	500
B-Cereals					
1	Maize (green)	5	3560	50	3510

Sl. No	Crop	Sale Price (Rs/Kg)	Gross extra income (Rs.) due to BF use/200m^2	Cost of BFs (Rs.) used)	Net Income (Rs./200m^2)
C- Pulses					
1	Greengram (Own consumption)	50	180	25	155
D-Oilseeds					
1	Soybean		Crop suffered drought.		
2	Sunflower	40	160	50	110
3	Sesamum	50	135	25	110
E- Fruits					
1	Banana(1000m^2)	13	9750	200	9550
F-Fibre crops					
1	Cotton(200m^2)	50	3750	100	3650
Total area – 6600m^2				**1050/-***	**42,855/-**

* Excluding cost of Vermicompost (as it is self generating source).

Annexure-I

List of Investigators: AINP on Soil Biodiversity-Biofertilizers

Sr.no	Centre	Name of P.I
1	AAU, Jorhat	Dr. D.J.Nath, Assoc. Professor
2	ANGRAU, Amaravathi	Dr. N. Trimurtulu, Pr. Scientist
3	BAU, Ranchi	Dr. Himanshu Dubey, Assoc. Professor
4	HAU, Hisar	Dr. Rajesh Gera, Professor
5	JNKVV, Jabalpur	Dr. A.K. Rawat, Professor and Head/ Dr. N. Gopal Mitra, Professor
6	KAU, Thrissur	Dr. D. Girija, Professor
7	KAU, Vellayani	Dr. K. S. Meenakumari, Professor
8	MAU, Parbhani	Dr. Syed Ismail, Assoc. Professor
9	MPUAT, Udaipur	Dr. Devendra Jain Asst. Professor
10	OUAT, Bhubaneswar	Dr. S.K. Pattanayak, Professor
11	RAU, Pusa	Dr. M.N.Jha, Professor and Head
12	TNAU, Coimbatore	Dr.D.Balachandar, Professor; Dr. M. Gnanachitra/Dr. R Parimaladevi, Asst. Professor
13	YSPUHF, Solan	Dr. Rajesh Kaushal, Assoc. Professor Dr. Anjali Chouhan, Asst. Professor
14	CRRI, Hazaribagh	Dr. D.Maiti, Pr. Scientist
15	University of Delhi	Dr. Ruplal, Professor
16	GBPUAT, Pantnagar	Dr. K.P. Raverkar, Professor
17	IARI, New Delhi	Dr. A.K.Saxena, Pr. Scientist and Head
18	DGR, Junagadh	Dr. K.K.Pal, Pr. Scientist
19	UAS, Dharwad	Dr. P.U.Krishnaraj, Pr. Scientist and Head
20	Coordinating Unit, IISS, Bhopal	Dr. D.L.N.Rao, Project Coordinator Ms. T.K.Radha, Scientist.

Annexure-II

Details of Manpower (Centre-wise)

Sr No	Centre	Prof/ Pr. Sci	Assoc. Prof /Sr.Sci	Asst. Prof /Sci	Tech. Asst.	Beldar/ Lab. Attndt.	Admin.	Total posts
1	AAU, Jorhat	-	1	1	1	1	-	4
2	ANGRAU, Amaravathi	-	1	-	1	1	-	3
3	BAU, Ranchi	-	-	1	-	-	-	1
4	HAU, Hisar	-	1	1	1	1	-	4
5	JNKVV, Jabalpur	-	1	-	1	1	-	3
6	KAU, Thrissur	-	-	1	-	-	-	1
7	KAU, Vellayani	-	-	1	-	-	-	1
8	MAU, Parbhani	-	1	1	1	1	-	4
9	MPUAT, Udaipur	-	-	1	-	-	-	1
10	OUAT, Bhubaneswar	-	1	-	1	1		3
11	RAU, Pusa	-	-	1	1	1		3
12	TNAU, Coimbatore	-	1	1	1	1	-	4
13	YSPUHF, Solan	-	1	1	1	1	-	4
14	CRRI, Hazaribagh		-	-	-	-	-	-
15	University of Delhi	-	-	-	-	-	-	-
16	GBPUAT, Pantnagar	-	-	-	-	-	-	-
17	IARI, New Delhi	-	-	-	-	-	-	-
18	DGR, Junagadh	-	-	-	-	-	-	-
19	UAS, Dharwad	-	-	-	-	-	-	-
20	Coordinating Unit, IISS, Bhopal	1(PC)	-	1	1	1	1*	5
	Total	1	8	11	10	10	1	**41**

Annexure-III

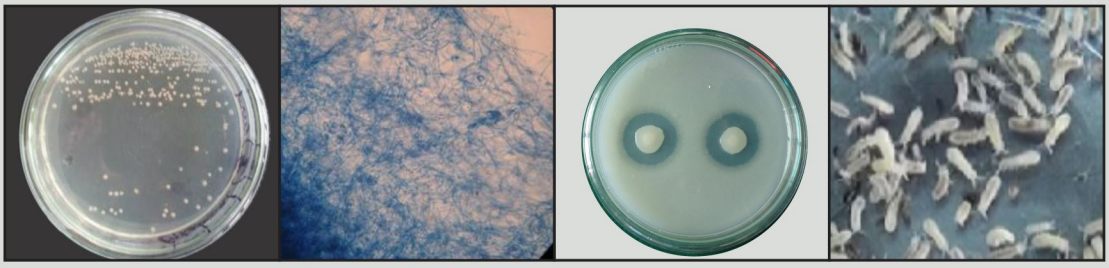
Centre-Wise Break-up of Budget (Rs. in lakhs)

Sr. no.	Centre	2014-15	2015-16
1	AAU, Jorhat	10.00	25.20
2	ANGRAU, Amaravathi	21.20	26.55
3	BAU, Ranchi	8.60	12.30
4	HAU, Hisar	17.50	19.80
5	JNKVV, Jabalpur	21.65	29.45
6	KAU, Thrissur	-	14.40
7	KAU, Vellayani	-	14.75
8	MAU, Parbhani	16.00	29.40
9	MPUAT, Udaipur	2.80	3.25
10	OUAT, Bhubaneswar	15.80	16.50
11	RAU, Pusa	6.30	14.80
12	TNAU, Coimbatore	18.30	33.90
13	YSPUHF, Solan	18.30	27.70
14.	CRRI, Hazaribagh	5.30	6.60
15	University of Delhi	5.30	5.40
16	GBPUAT, Pantnagar	-	7.40
17	IARI, New Delhi	4.30	2.20
18	DGR, Junagadh	4.30	7.40
19	UAS, Dharwad	5.30	4.60
20	Coordinating Unit, IISS, Bhopal	4.10	8.00
	Total	185.0	309.6

Annexure-IV

Recommendations of Group Meeting of ICAR All India Network Project on Soil Biodiversity- December 6-8, 2014, DGR, Junagadh, Gujarat,

1. For molecular analysis of rhizobial diversity all centres may complete depositing well characterized rhizobial isolates at DGR. Work on diversity of rhizobia of pulses and other legumes to be completed in 2015-16 (DGR, Junagadh).
2. Nodulation surveys in arid zone to be repeated 4-5 weeks after commencement of rain. Market survey on quality of biofertilizers be repeated. Best isolates of cowpea, pigeon pea and cluster bean rhizobia be promoted for biofertilizer production (HAU, Hisar).
3. Development of suppressive soils using plant growth promoting and DAPG-producing fluorescent pseudomonads be done. Fluorescent pseudomonads be evaluated for disease suppression in groundnut in farmers fields (DGR, Junagadh).
4. Diversity of endophytic microbiome of groundnut may be studied under moisture deficit and salinity stress. Consortia of compatible PGPR, PSM and rhizobia tolerant to low moisture, salinity and high temperature be developed for alleviation of abiotic stresses in groundnut (DGR, Junagadh)
5. Symbiotic efficiency of rhizobial isolates be tested against candidate crops (MPUAT, Udaipur)
6. Nodulation test for pigeonpea may be repeated in cups/leonard jars. For comparing differences in the molecular characteristics of the spots on 2D protein gels, isolates of rhizobia may be classified into slow growing/fast growing, cross-inoculation groups or acid-tolerant and acid-sensitive ones (BAU, Ranchi).
7. Various genotypes of *Phaseolus* be used as trap plants to tap the full range of rhizobial diversity. MPN counts of rhizobia to be done in both rhizosphere and non-rhizosphere soil (GBPUAT, Pantnagar)
8. Explore the possibility of testing the effect of inoculating rhizobia antagonistic to plant pathogenic fungi in sick plots for testing plant resistance against *Fusarium.* The functional analysis of candidate genes may be done in the best strains (IARI).
9. Soils under INM practice be included for metagenomic analysis (UAS).
10. Selected isolates of *Pseudomonas* be sequenced for comparative genomic analysis (DU).
11. Study the impact of actinomycetes on other beneficial microflora (*Azotobacter*, *Azospirillum*, PSB) (JNKVV)
12. The research mandate may be concentrated on fewer crops (YSPUHF).
13. Endophyte showing similarity with *Piriformospora indica* – may be got identified (ANGRAU).
14. Full length sequencing of 16S rRNA gene be done for the PGPR isolates (KAU, Thrissur).
15. Quantification of P, K, & Zn solubilisation in the rhizosphere may be done after microbial inoculation (AAU, MKV).
16. Best cultures from other centres may be tested for comparison. Other treatments such as incubated FYM only (without cultures) may be included (OUAT)
17. Conduct composting technology demonstration among tribal farmers (KAU, Vellayani).

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