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Dear Reader,

You are well aware that currently a new science has emerged, called as “Sustainability Science” which is multi disciplinary and multi dimensional. To address each area of human interference in nature, the technology, which can help to achieve the desired goal without harming ecological system, which is the basics of sustainable science. In modern agriculture, the thrust has been to achieve an ever green revolution which can ensure enhancement of productivity with perpetuity. Conservation farming and green agriculture which involve the use of integrated natural resources management techniques are the path ways for ever green revolution. Under such scenario, use of biofertilizers offers high potentialities for their use to supplement crop nutrients for optimizing crop productivity besides maintaining soil health and fertility which is the key for ever green revolution.

In order to optimize, the use of biofertilizers has been the one of the endeavors of the National Project of Organic Farming which is presently perused by this Department. Scrutiny of literature indicate, India has been utilizing biofertilizers like *Azotobacter*, *Azospirillum*, *Rhizobium*, *Cyanobacteria*, *Azolla*, *Phosphate Solubilizing Bacteria* and *Mycorrhiza* in large quantity to supplement crop nutrients in agricultural production. Although, India has emerged as one of the largest Biofertilizer producing country in the globe but its quality is poor in terms of microbial count and high contamination as compared to developed countries. Accordingly, development of new inoculants formulations and technology for increasing shelf life of micro-organisms is very important and this issue has incorporated for use by commercial inoculants producers. This issue also has focused use of arbuscular mycorrhizal fungi (AMF) in the economically important crop like ginger, whose organic demand is high. Moreover, this issue has highlighted recent news mostly on development of inoculant technology inform of research Notes and New Reports, Seminar/Conference, Symposium news and Book Reviews.

Hope, this issue would fulfill the aspirations of our esteemed readers.

Dr. R.N. Bisoyi
Editor

***Glomus* spp. – A Suitable Plant Growth Promoting Fungus for Growth and Establishment of Tissue Culture Derived Ginger**

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Introduction

One of the important applications of biotechnology in agriculture is its use in the micro propagation of plants. Micro propagation techniques are increasingly being applied for large-scale production of quality planting material. Ginger is one of the economically important spices of Kerala with tremendous export potential. The ginger plantlets normally take two years to yield and show delayed rhizome formation. It is estimated that in general, only about 25 per cent of *in-vitro* generated plantlets are successfully transplanted *ex-vitro* and still fewer transferred to the field. Moreover, it takes approximately 8-12 months to produce plantlets large enough to withstand the rigors of nursery conditions and the acclimatization phase of tissue cultured (TC) plants undergoes a lag phase before they are able to establish in the field.

The plant growth promoting fungus (PGPF) are abundant in the rhizosphere region, which produce various growth promoting substances that promote growth of roots, absorb sufficient nutrients and increase the activity of beneficial organisms in the rhizosphere. As a result, PGPF enhances the plant growth, control soil borne diseases and also induce systemic resistance to phytopathogen. The PGPF normally includes Mycorrhiza and *Trichoderma* spp. The AMF naturally exist in different cropping ecosystems and enrichment of

their population in the rhizosphere profoundly increase plant growth in a wide range of crops (Podile and Krishnakishor, 2003). Mycorrhiza is also used for the growth and establishment of tissue culture derived plants which will have beneficial effects like improved water relations, uptake of nutrients and tolerance to pathogenic infection, which in turn will result in enhanced growth, survival and establishment of plantlets. Hence, a study was conducted to find a suitable arbuscular mycorrhizal fungus for the growth and establishment of micro-propagated ginger.

Materials and Methods

- a. **Survey of ginger growing Areas in Kerala State** - The major ginger growing areas in three locations of each district were identified. A total of 15 locations were selected for rhizosphere soil sample collection and were used for the isolation of arbuscular mycorrhizal fungi.
- b. **Isolation, Identification and Mass Multiplication of Arbuscular Mycorrhizal Fungi** - The wet sieving and decanting method was used for the isolation of AM fungal spores from the rhizospheric soil of ginger, and the most predominant spores from each soil sample collected from different ginger growing areas were mass multiplied using standard protocols.
- c. **Screening of different isolates** - A total of 15 isolates of AMF were obtained from various ginger growing

areas of Kerala and were screened against growth and establishment of TC derived ginger under sterile pot culture experiment. A potting mixture consisting of sand: soil: dried cow dung (1:1:1 ratio) was prepared and sterilized in an autoclave for 2 hours on three consecutive days. After sterilization, the potting mixture was allowed to cool at room temperature. The sterilized potting mixture was filled in micro pots (7 cm x 6.5 cm). The experiment was laid in completely randomized design. The *in-vitro* derived *ex-agar* plants of ginger were obtained from CPBMB, College of Horticulture and used for the study. The plantlets were taken out from the test tubes and roots were washed till the agar was removed and then roots were dipped in Indofil fungicide for 10 min. The ginger plantlets were planted in a micro pots (7 x 6.5 cm) containing sterilized sand. The plants were covered with polythene bags (with holes) and after one month of planting, the plants were transplanted to the pots containing sterile potting mixture. The substrate based bulk inoculum of AMF was placed @ 50 g / plant near root zone at the time of planting. The observations on survival, plant height (cm), number of tillers, dry weight of plants and rhizome yield were recorded up to 5 months after planting. The data were analysed by Kruskal-Wallis test using SPSS software.

Result and Conclusions

Identification of AMF

The different isolates of AM fungi obtained from rhizosphere soil of ginger growing areas or Kerala were identified using standard keys. The isolates were globose, light brown, smooth surface configuration with straight hyphae and these characters were compared with standard keys as well as INVAM website. All the isolates were tentatively identified as *Glomus* spp (Table 1). The

AMF population ranged from 53-200 per 50 g of soil sample.

Screening of isolates

The effect of different isolates of AMF on growth and establishment of ginger revealed maximum plant height in the case of AMF11 isolate (54.5 cm) followed by AMF9, isolate (54 cm) (Table 2). In the case of plant dry weight, the maximum dry weight was recorded with AMF9 (138.9 g / plant) followed by AMF1 (118.6 g/ plant). Maximum rhizome yield was recorded with AMF3 (111.35 g/plant) followed by AMF 13 (92.01g/plant.) Based on different biometric characters and rhizome yield, *Glomus* sp. (AMF3) was found to be most effective in enhancing the growth and establishment of micro-propagated ginger. There are several studies to prove the efficiency of AMF in enhancing the growth of various crops. The rhizosphere fungi are not only successful in biocontrol of plant diseases but many of these particularly AMF and *Trichoderma* spp. plays an important role in promotion of plant growth and yield (Sivaprasad, 2002). Jayanthi and Bagyaraj (1998) reported that *Glomus mosseae* fungus inoculated singly with various combinations with *Bacillus coagulans* and *Trichoderma harzianum* resulted in enhanced growth and nutrition of micro propagated sugarcane plantlets. Adding arbuscular mycorrhizal fungi, ericoid or arbutrioid mycorrhizal fungi into the rooting medium of micro-propagated plants produced better rooted cutting that were better able to withstand the stress of transplanting (Scagel, 2001). The present study clearly indicated the efficiency of *Glomus* sp. (AMF3 isolate). However, extensive field trials are needed to test the efficiency under different edaphic factors so as to commercialize the inoculum production.

References

- Jayanthi S and Bagyaraj DJ (1998). Response of micro propagated sugarcane to *Glomus mosseae*, *Trichoderma harzianum*, and *Bacillus coagulans*. *J. Soil. Biol. Ecol.* 18 . 23-29.
- Podile A R and Krishnakishore G (2003). Dual inoculation of rhizobia and PGPR inoculants increase the productivity of legume crops. *Souvenir of 6th International workshop on Plant Growth Promoting Rhizobacteria*. Indian Institute of Spices Research, Calicut, 2003. .56-61 pp.
- Sivaprasad P (2002). Potential of bioinoculants for sustainable agriculture. In: proceedings and recommendations of Research Extension interface. Kerala Institute of Local Administration, Trichur. .24-30 pp
- Scagel Crolyn F (2001). Stimulation of adventitious rooting on cuttings from woody perennial plants by exposure to inoculum to ericoid and arbuscular mycorrhizal fungi. In: *Proceedings of 3rd international, conference on Mycorrhizas. Adelaide, Australia.* 1-15

A rhizosphere perspective on inoculant efficacy

The presence of the root defines the rhizosphere, while the intimate interactions between the plant and soil biota within the soil habitat characterize the rhizosphere. Root exudates are the substrate or fuel for the intense microbial (bacteria, fungi, algae, protozoa, nematodes and arthropods) activity within the rhizosphere. Thus, it is the quantity and quality of the exudates and condition of the soil habitat will determine the colonization potential of the rhizosphere. Each plant species leaks a unique carbon and nitrogen signature of carbohydrates, amino acids and organic acids that determines the primary colonizers of the microbial community. The composition of the exudates also affects the availability of nutrients in the immediate vicinity of the roots influencing the establishment and growth of the plant. An increase or decrease in the nutritional status of the plant can further alter the quality of the exudates affecting the microbial diversity and populations in the rhizosphere. Root biomass and architecture, fertiliser type, tillage and cropping history also affect root growth and patterns of exudation and the populations and diversity of rhizosphere colonizing organisms. Together, these factors determine whether rhizosphere interactions and processes will have a positive, neutral or beneficial affect on plant growth. Microbial inoculants placed with or in the immediate proximity of the seed must be able to successfully establish and multiply along the growing root, and integrate into the rhizosphere community or alter and/or overwhelm the community to give the desired growth effect. The diversity of crops in a rotation, perhaps even the sequence of crops in a rotation not to mention the cultivar will affect the efficacy of an inoculant regardless of the crop and soil management techniques. It is suggested that the greatest success will be achieved in developing inoculants that contain cool temperature competitive strains, and a diversity of species that are more tailored to a particular crop species or type. Technical considerations aside, one size fits no one. There is also a need to work with plant breeders to develop appropriate inoculants, that work in synergy with the crop traits, particularly rooting

Improvement in Formulations and Shelf Life of Consortia of Microorganisms

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The importance of microorganisms in biological nutrient mobilization and plant growth promotion are well known and various microorganisms singly or in combinations are being exploited as biofertilizers. Biofertilization now is a mature biotechnology. The journey started with *Rhizobium* now encompasses long list of microorganisms with most important being *Azotobacter*, *Azospirillum*, *Acetobacter*, *Rhizobium* and Phosphate solubilizers such as *Bacillus polymyxa*, *Bacillus megaterium*, *Bacillus subtilis*, *Pseudomonas striata*, *Aspergillus awamorii*, *Penicillium digitatum* etc. Recently another group of microorganisms known as Plant Growth Promoting Rhizobacteria (PGPR) such as *Pseudomonas fluorescens* and Mycorrhizal fungi such as *Glomus* spp have also attracted lot of attention. So far commercial biofertilizers are mono strain bacterial/ fungal cultures. Large numbers of co-inoculation studies have proved that use of multiple microorganisms give much higher benefit compared to single microorganism. To develop suitable consortia of such beneficial microorganisms in appropriate formulation with sufficient shelf life, efforts were made under "All India Network Project on Biofertilizers" (AINPB). Abstract details of the formulation medium and strength of different microorganisms during the period of shelf life are presented here.

Formulation of common co-culture medium - Experimental and field studies conducted under AINPB on different crops revealed that mixed application of *Azospirillum lipoferum*, *Bacillus megaterium* and *Pseudomonas fluorescens* not only gives best result in terms of overall crop growth and yield but these microorganisms are also compatible to each other when formulated into mixed inoculant. Based on the nutritional properties of the organisms in the inoculant formulations a basic medium which can support the growth of all the three organisms was formulated. Final composition of the co-culture medium was standardized after population count of the organisms and adjusting the concentration of media contents accordingly. Among the different concentrations of media tested M-40 supported the good growth of all the microorganisms under study and was considered as a new co-culture medium (CCM-B).

Shelf life of co-cultured inoculants in carrier and liquid formulations- Population dynamics of *Azospirillum*, *Pseudomonas* and *Bacillus* in carrier as well as in liquid formulation was studied at monthly intervals for six months. The results were compared with the respective single cultures. As general rule, individually grown inoculants recorded higher population than the co-cultured inoculants. In co-cultured liquid inoculant formulation at 6 months counts of *B. megaterium* were 3.0×10^8 , *P. fluorescens* 5.3×10^7 and *A. lipoferum*

4.3x10⁷ CFU ml⁻¹ (Table 2). In Liquid inoculant formulation, addition of PVP at 2% level maintained the population of *A. lipoferum* and *P. fluorescens* at 10⁹ cfu ml⁻¹ level upto 5 months period and *B. megaterium* populations at 10⁹ cfu ml⁻¹ up to 6 months of preparation (Table 3).

Table 1 Microbial counts in various co-culture media formulations

Media	Cell count in the inoculant (CFU ml ⁻¹)		
	<i>A. lipoferum</i>	<i>B. megaterium</i>	<i>P. fluorescens</i>
M 39	0.43x10 ⁷ (7.63)	8.33 x10 ⁹ (9.92)	6.0 x10 ⁸ (8.78)
M 40	0.75 x10 ⁷ (7.88)	7.00 x10 ⁹ (9.85)	8.66 x10 ⁸ (8.94)
M 41	0.44 x10 ⁶ (6.64)	3.66 x10 ⁹ (9.56)	9.66 x10 ⁷ (7.98)
M 42	0.12 x10 ⁷ (7.08)	7.66 x10 ¹⁰ (10.88)	5.00 x10 ⁸ (8.70)
M 43	0.20 x10 ⁶ (6.30)	7.33 x10 ¹¹ (11.87)	9.66 x10 ⁷ (7.98)
SEd	0.69	0.96	0.82
LSD (p=0.05)	1.55	2.14	1.82

Table 2. Cell counts of co-cultured inoculants (CCM-B medium) in carrier based formulations.

Time (months)	Cell counts Expressed as log-transformed values					
	Single culture			Co-culture		
	<i>A. lipo.</i>	<i>B. mega.</i>	<i>P. fluor.</i>	<i>A. lipo.</i>	<i>B. mega.</i>	<i>P. fluor.</i>
1	9.81	10.97	10.85	8.88	9.88	9.92
2	9.72	10.88	9.92	8.81	9.80	8.94
3	8.88	10.48	9.70	8.63	9.30	8.80
4	8.72	9.92	9.48	8.36	8.87	8.37
5	8.64	9.70	9.00	7.81	8.70	7.88
6	8.56	9.56	8.94	7.63	8.48	7.73
SEd	0.91	0.90	0.90	0.80	0.80	0.80
LSD (p=0.05)	2.0	1.9	1.9	1.8	1.7	1.7

Table 3. Microbial counts in liquid inoculant with different additives after 6 months of storage.

Additives	Cell count in the inoculant (CFU ml ⁻¹)		
	<i>A. lipoferum</i>	<i>A. lipoferum</i>	<i>A. lipoferum</i>
T1 Glycerol (1%)	7.5x10 ⁴ (4.88)	4.00x10 ⁸ (8.60)	8.0x10 ⁶ (6.90)
T2 Glycerol (2%)	4.3x10 ⁵ (5.63)	5.33x10 ⁸ (8.73)	9.32x10 ⁸ (6.97)
T3 PVP (1%)	8.0x10 ⁸ (8.90)	6.0x10 ⁹ (9.78)	7.00x10 ⁸ (8.85)
T4 PVP (2%)	9.3x10 ⁸ (8.97)	7.0x10 ⁹ (9.85)	9.0x10 ⁸ (8.95)
T5 Sod. lutamate	7.5x10 ³ (3.88)	3.0x0 ⁷ (7.48)	7.33x10 ⁵ (5.33)
T6 Trehalose 5mM	1.4x10 ⁷ (7.15)	1.33x10 ⁸ (8.12)	5.33x10 ⁶ (6.73)

Research Notes, New Reports

The Study of Shelf Life for Liquid Biofertilizer from Vegetable Waste -

Liquid biofertilizer are increasingly available in the market as one of the alternatives to chemical fertilizer and pesticides. One of the benefits from biofertilizer is a contribution to increased population of microorganisms. Traditionally, liquid biofertilizer produced from fermentation of effective microorganisms (EM) was recommended for use within three months. The experiment showed that shelf life of the liquid biofertilizer produced from vegetable waste contained high amount of viable microbial population after four months of storage. The two conditions of storage, with and without light, were tested and it was found that there was no significant difference ($p > 0.05$) upon viable microbial population, chemical and physical characteristics. However, there was significant difference from batch to batch of production due to differences in raw materials. (Source-Hasarin et al 2008 Australian J Technology 11(4): 204-208)

Recent phycotechnological advances concerning bio-N fertilization of rice -

The flooded water and the surface of the soil provide the sites for aerobic phototrophic nitrogen fixation by free living cyanobacteria and *Azolla-Anabaena* symbiotic N_2 -fixing complex. The free-living cyanobacteria, majority of which are heterocystous and nitrogen-fixing, contribute on an average 20-30 kg $N\ ha^{-1}$, while the value is up to 60 kg ha^{-1} in the case of *Azolla-Anabaena* system. Synthesis and excretion of organic/growth promoting substances by the cyanobacteria are also on record. During the last 2-3 decades, a great volume of work has been accomplished on the various important fundamental

and applied aspects of both kinds of cyanobacterial biofertilizers, which include: strain identification, isolation, purification and culture; laboratory analyses of their N_2 -fixing activity and related physiology, biochemistry and energetics; identification of the structure and regulation of nitrogen fixing (*nif*) genes and nitrogenase enzyme; symbiotic biology of *Azolla-Anabaena* mutualistic N_2 -fixing complex; free living cyanobacterial strain improvement through mutagenesis with respect to constitutive N_2 fixation and resistance to the non-congenial agronomic factors; preliminary meristem mutagenesis in *Azolla* for achieving reduced phosphate dependence, temperature tolerance and significant sporulation/spore germination under controlled conditions; mass production biofertilizer technology of free-living and symbiotic (*Azolla-Anabaena*) cyanobacteria; interacting and agronomic effects of both kinds of cyanobacterial biofertilizer with rice; and economics of rice cultivation with the cyanobacterial biofertilizers. While the research, so far, are an indicative of the strong potential of cyanobacterial biofertilizer technology in rice growing countries, open up vast area of more concerted basic, applied and extension work in future to make these self-renewable natural nitrogen resources further promising at field level in order to help reducing the requirement of inorganic N to be the bare minimum, if not zero. (Source-Vaishampayan in Biofertilizers technology for rice based cropping system Advances in plant Physiology vol 4, No.1(2002)

Interaction Effect of some Biofertilizers and Irrigation Water Regime on Mung bean (*Vigna radiata*)

Growth and Yield - The interactive effect of biogin and nitrobin biofertilizers and compost on growth, yield and metabolic products of mung bean (*Vigna radiata* L.) under different irrigation water regimes of 5d, 10d & 15d drying cycle were studied. Growth and yield were suppressed in plants of water regime II (10d drying cycle), while plants under regime III (15d drying cycle) were severely and harmfully affected. Addition of biofertilizers mitigated the harmful effect of water stress. The greatest yield/plant was obtained in plants of water regime I (5d drying cycle) treated with biogin amounting to 282% of unfertilized plants. Plants treated with biogin under regime II (10 d drying cycle) yielded 145% of unfertilized control (regime I). Biofertilized plants exhibited higher values of leaf metabolic products than non-fertilized plants. The response of leaf metabolic products to nitrobin was more marked than for the other biofertilizers. Commonly slightly higher amounts of total carbohydrates, lipid and total crude protein contents were obtained in seeds of plants irrigated every 5 d than those of 10 d drying cycle while they were more or less comparable for the biofertilized and nonfertilized plants. Plants treated with nitrobin produced the highest level of genistein (isoflavonoid) under water regime I or II and the least level of quercetin (flavonoid). (Source-Sheteawi, and Tawfik, 2007 Journal of Applied Sciences Research, 3(3): 251-262)

Biofilmed biofertilisers: Novel inoculants for efficient nutrient use in plants -Microbial communities attached to surfaces, or biofilms, are found in many environments, including the soil. This chapter describes the potential applications of developed biofilms as biofertilisers in crop production. Formation of fungal–bacterial biofilms (FBBs) by bacterial colonisation on

biotic fungal surfaces gives the biofilms enhanced metabolic activities compared to monocultures. Incorporation of a nitrogen (N₂)-fixing rhizobial strain to FBBs to form fungal–rhizobial biofilms (FRBs) has been shown to improve potential biofilm applications in N-deficient settings and in the production of biofilmed inocula for biofertilisers and biocontrol in plants. A developed biofilmed inoculant of the FRB significantly increased N₂ fixation in soybean by ca. 30% compared to a conventional inoculant of rhizobium alone (monoculture inocula). The FBB and FRB increased biomass in early growth of rice by ca. 25% compared to the conventional inocula. Root colonisation of wheat by FRBs forming ‘pseudonodules’ was also observed recently. The FRBs increased N and phosphorus (P) availabilities when inoculated directly to the soil. They also improved P biosolubilisation from rock phosphate. The FBBs of beneficial endophytes produced higher acidity and plant growth promoting hormones than their mono- or mixed cultures with no bio film formation. This indicates that the highest microbial effect may not be achieved by plant inoculation with the conventional inocula of effective microbes, but only by bio filmed inoculate. (Source-Gamini et al ICIAR Research that works for developing countries and Australia, ACIAR, Sept 2008, pg126-130)

Characterization of *Rhizobium* strain isolated from the roots of *Trigonella foenumgraecum* (fenugreek) - *Trigonella foenumgraecum* (fenugreek) is known for its dietary protein source, medicinal properties and symbiotic nitrogen fixation by *Rhizobium* present in its root nodules. The present study describes the characterization of a *Rhizobium* strain isolated from root nodules of fenugreek. The *Rhizobium* isolates were rod shaped, gram

negative, acid and mucous producing. They were found to be temperature and pH sensitive, with optimum values of 29.4 and 7.0°C, respectively. The bacteria was sensitive to the antibiotics; chloramphenicol, kanamycin and streptomycin. It utilizes glucose, sucrose and starch as sole carbon source. The *Rhizobium* species isolated from fenugreek roots have the potential to produce industrially important enzymes; amylase and cellulase. Immobilizing the organism in agar and agarose does not affect its activity; indeed increased biomass yield and enzyme production was observed. The *Rhizobium* can be easily immobilized onto carriers like charcoal powder which can be applied as biofertilizer. (Singh et al 2008, African Journal of Biotechnology Vol. 7 (20) - 3671–3676)

Synergistic effects of plant growth promoting rhizobacteria and *Rhizobium* on nodulation and nitrogen fixation by pigeonpea (*Cajanus cajan*) - Plant-growth promoting rhizobacteria (PGPR), in conjunction with efficient *Rhizobium*, can affect the growth and nitrogen fixation in pigeon pea by inducing the occupancy of introduced *Rhizobium* in the nodules of the legume. This study assessed the effect of different plant-growth promoting rhizobacteria (*Azotobacter chroococcum*, *Azospirillum brasilense*, *Pseudomonas fluorescens*, *Pseudomonas putida* and *Bacillus cereus*) on pigeon pea (*Cajanus cajan* (L) Milsp.) cv. P-921 inoculated with *Rhizobium* sp. (AR-2–2 k). A glasshouse experiment was carried out with a sandy-loam soil in which the seeds were treated with *Rhizobium* alone or in combination with several PGPR isolates. It was monitored on the basis of nodulation, N₂ fixation, shoot biomass, total N content in shoot and legume grain yield. The competitive ability of the introduced *Rhizobium* strain was assessed by calculating nodule

occupancy. The PGPR isolates used did not antagonize the introduced *Rhizobium* strain and the dual inoculation with either *Pseudomonas putida*, *P. fluorescens* or *Bacillus cereus* resulted in a significant increase in plant growth, nodulation and enzyme activity over *Rhizobium*-inoculated and uninoculated control plants. The nodule occupancy of the introduced *Rhizobium* strain increased from 50% (with *Rhizobium* alone) to 85% in the presence of *Pseudomonas putida*. This study enabled us to select an ideal combination of efficient *Rhizobium* strain and PGPR for pigeon pea grown in the semiarid tropics. (Source - K. V. B. R. Tilak. E-mail: tilakkvbr@yahoo.com)

Diversity of plant growth and soil health supporting bacteria - The global necessity to increase agricultural production from a steadily decreasing and degrading land resource base has placed considerable strain on the fragile agro-ecosystems. Current strategies to maintain and improve agricultural productivity via high input practices place considerable emphasis on 'failsafe' techniques for each component of the production sequence with little consideration to the integration of these components in a holistic, systems approach. While the use of mineral fertilizers is considered the quickest and surest way of boosting crop production, their cost and other constraints deter farmers from using them in recommended quantities. In recent years, concepts of integrated plant nutrient management (IPNM) have been developed, which emphasize maintaining and increasing soil fertility by optimizing all possible sources (organic and inorganic) of plant nutrients required for crop growth and quality. This is done in an integrated manner appropriate to each cropping system and farming situation. Improvement in agricultural sustainability requires optimal use and management of soil fertility and soil

physical properties, both of which rely on soil biological processes and soil biodiversity. An understanding of microbial diversity perspectives in agricultural context, is important and useful to arrive at measures that can act as indicators of soil quality and plant productivity. In this context, the long-lasting challenges in soil microbiology are development of effective methods to know the types of microorganisms present in soils, and to determine functions which the microbes perform *in situ*. This review describes some recent developments, particularly in India, to understand the relationship of soils and plants with the diversity of associated bacteria, and traces contributions of Indian scientists in isolating and defining the roles of plant growth promoting bacteria to evolve strategies for their better exploitation. (Source – Tilak et al 2007, Current Science Vol 89 - 283–292)

Effect of Biofertilizers and Humic Acid on control of Dry Root Rot Disease and Improvement in Yield Quality of Mandarin (*Citrus reticulata* Blanco) -

The effect of soil drenching with humic acid and commercial biofertilizers *i.e.*, phosphorien , microbien and/or cearalien were evaluated for controlling dry root rot disease of mandarin ,fruit quality and total yield during 2005 and 2006seasons . *In vitro* , humic acid at 15.0%(v/v) reduced significantly the radical growth and spore germination of *Fusarium solani* the causal agent of dry root rot . Drenching artificially infested soil with humic acid +Phosphorien or microbien biofertilizers as twice applications resulted in decrease of both disease infection and severity on mandarin seedlings as well as reduce colonization of *F. solani* in seedlings roots. In Field trials, humic acid +phosphorien or microbien biofertilizer treatments were significantly increased the number of recovering diseased trees , decrease the disease severity on the others, inhibiting

the activity of the pathogen in rhizosphere soil of treated trees . Moreover, these treatments improved the yield and fruit quality also of treated trees . Therefore, the usage of combination between humic acid and biofertilizers could be suggested as a bio-treatment for controlling dry root rot of citrus especially under organic system . (Source - El-Mohamedy and Ahmed Res. J. Agric. & Biol. Sci., 5(2): 127-137, 2009).

Effects of Azospirillum brasilense indole-3-acetic acid production on inoculated wheat plants -

The production of phytohormones by plant-growth promoting rhizobacteria is considered to be an important mechanism by which these bacteria promote plant growth. In this study the importance of indole-3-acetic acid (IAA) produced by *Azospirillum brasilense* Sp245 in the observed plant growth stimulation was investigated by using Sp245 strains genetically modified in IAA production. Firstly wild-type *A. brasilense* Sp245 and an ipdC knock-out mutant which produces only 10% of wild-type IAA levels were compared in a greenhouse inoculation experiment for a number of plant parameters, thereby clearly demonstrating the IAA effect in plant growth promotion. Secondly, the question was addressed whether altering expression of the ipdC gene, encoding the key enzyme for IAA biosynthesis in *A. brasilense*, could also contribute to plant growth promotion. For that purpose, the endogenous promoter of the ipdC gene was replaced by either a constitutive or a plant-inducible promoter and both constructs were introduced into the wild-type strain. Based on a greenhouse inoculation experiment it was found that the introduction of these recombinant ipdC constructs could further improve the plant-growth promoting effect of *A. brasilense*. These

data support the possibility of constructing *Azospirillum* strains with better performance in plant growth promotion. (Source – Spaepen et al 2008 Plant and Soil 312 (1-2) 15-23)

Alternatives to peat as a carrier for rhizobia inoculants: Solid and liquid formulations

- Many of the microbial inoculants all over the world are based on solid peat formulations. This has been mostly true for well developed legume inoculants based on selected rhizobial strains, due to peat bacterial protection properties. Six carriers (bagasse, cork compost, attapulгите, sepiolite, perlite and amorphous silica) were evaluated as alternatives to peat. Compost from the cork industry and perlite were superior to peat in maintaining survival of different rhizospheric bacteria. Other tested materials were discarded as potential carriers for soybean rhizobia. Also, different liquid culture media have been assayed employing mannitol or glycerol as C sources. Some media maintained more than 10^9 cfu ml⁻¹ of *Sinorizobium Ensifer*) *fredii* SMH12 or *Bradyrhizobium japonicum* USDA110 after 3 months of storage. Rhizobial survival on pre-inoculated seeds with both solid and liquid formulations previously cured for 15 days led to a higher bacterial numbers in comparison with recently made inoculants. An additional curing time of solid inoculants up to 120 days had a beneficial effect on rhizobial survival on seeds. The performance of different formulations of two highly effective soybean rhizobia strains was assayed under field conditions. Soybean inoculated with cork compost, perlite and liquid formulations produced seed yields that were not significantly different to those produced by peat-based inoculants (Source – Albareda et al 2008 Soil Biology and Biochemistry 40 (11), 2771-2779)

Field performance of a liquid formulation of *Azospirillum brasilense* on dryland wheat productivity

- The beneficial effects of inoculating with *Azospirillum brasilense* on crop productivity have been widely described, but extensive use in typical agricultural field environments is scarcely documented. The objective of this study was to quantify the productivity of wheat (*Triticum aestivum* L.) whose seed was inoculated with a liquid formulation containing *Azospirillum brasilense* INTA Az-39 strain under typical dryland farming conditions. The study was performed in the 2002–2006 growing seasons, evaluating inoculated and non-inoculated seed at 297 experimental locations in the Pampas region of Argentina. The inoculated crops exhibited more vigorous vegetative growth, with both greater shoot and root dry matter accumulation (12.9 and 22.0%, respectively). The inoculation increased the number of harvested grains by 6.1%, and grain yield by 260 kg ha⁻¹ (8.0%). Positive responses were determined in about 70% of the sites, depending mostly on the attainable yield and independently of fertilization and other crop and soil management practices. In general, more response to inoculation was observed in the absence of major crop growth limitations, suggesting the complementary contribution of the *Azospirillum brasilense* treatment to more efficiently developing higher yielding wheat. (Source – Diaz-Zorita et al 2009 European Journal of Soil Biology 45 - 3-11)

Effect of Furadan on the Growth and Nitrogen Fixation by Blue Green Algae

- The study was carried out to determine the effects of pesticide on the growth and nitrogen fixation by blue green algae (BGA) that isolated from three different soils

viz., saline soil, calcareous soil and red soil. Furadan 5G, one of the most commonly used pesticides was selected for the study of eighteen taxa of blue green algae (BGA) which brought under the unialgal culture in the selected soil types. Variations observed in growth and nitrogen fixation among the isolates of a particular type of soil. The culture resulted as BGA grow slow under heterotrophically grown cultures than that of autotrophically grown cultures. On the other hand, nitrogen fixation in heterotrophically grown cultures was observed at higher rate. However satisfactory result was observed in both cases when field dose of Furadan was applied practically. In this case, heterotrophically grown cultures were more tolerant to pesticides with respect to growth and nitrogen fixation. (Source – Islam et al 2007 Journal of Bio-Science, Vol 15)

PSM and *Azotobacter* Population in Rhizosphere Soil of Mustard as Influenced by Organic and Inorganic Sources - A field experiment was conducted during rabi of 1999-2000 on the PSM and *Azotobacter* population in the rhizosphere soil as influenced by biofertilizer, farmyard manure and chemical fertilizer. Data revealed that increasing fertilizer levels from 0 to 100% (based on soil test recommendation), farmyard manure at 10 t/ha and co-inoculation with *Azotobacter* and PSM significantly increased the PSM and *Azotobacter* population in rhizosphere soil. The study showed significant effect of interactions of fertilizer x biofertilizers, FYM x biofertilizers, fertilizers x FYM and fertilizers x FYM x biofertilizers on PSM and *Azotobacter* population. The maximum population was observed at highest level of each of the treatments indicating that native soil was having sub-optimum population of these

bacteria and responded to single and combined application with positive interaction. The significant and positive correlation of coefficient observed between *Azotobacter* and N uptake and PSM population and P uptake provides circumstantial evidence to the role of these microorganisms in improving crop productivity. (Source Chand and Ram 2008 Environment and Ecology Vol. 26-43-46)

Impact of antifungals producing rhizobacteria on the performance of *Vigna radiata* in the presence of arbuscular mycorrhizal fungi - Plant growth-promoting rhizobacteria (PGPR) that produce antifungal metabolites are potential threats for the arbuscular mycorrhizal (AM) fungi known for their beneficial symbiosis with plants that is crucially important for low-input sustainable agriculture. To address this issue, we used a compartmented container system where test plants, *Vigna radiata*, could only reach a separate nutrient-rich compartment indirectly via the hyphae of AM fungi associated with their roots. In this system, where plants depended on nutrient uptake via AM symbiosis, we explored the impact of various PGPR. Plants were inoculated with or without a consortium of four species of AM fungi (*Glomus coronatum*, *Glomus etunicatum*, *Glomus constrictum*, and *Glomus intraradices*), and one or more of the following PGPR strains: phenazine producing (P(+)) and phenazine-less mutant (P(-)), diacetylphloroglucinol (DAPG) producing (G(+)) and DAPG-less mutant (G(-)) strains of *Pseudomonas fluorescens*, and an unknown antifungal metabolite-producing *Alcaligenes faecalis* strain, SLHRE425 (D). PGPR exerted only a small if any effect on the performance of AM symbiosis. G(+) enhanced AM root colonization and had positive effects on shoot growth and nitrogen content when added alone, but

not in combination with P(+). D negatively influenced AM root colonization, but did not affect nutrient acquisition. Principal component analysis of all treatments indicated correlation between root weight, shoot weight, and nutrient uptake by AM fungus. The results indicate that antifungal metabolites producing PGPR do not necessarily interfere with AM symbiosis and may even promote it thus carefully chosen combinations of such bioinoculants could lead to better plant growth. (Source Dwivedi et al, DOI-10-1007/s00572-009-0253-2, May 21, 2009 Mycorrhiza.)

Growth of *Jatropha curcas* on heavy metal contaminated soil amended with industrial wastes and *Azotobacter* – A greenhouse study -

The aims of the study were to evaluate the effect of organic wastes (biosludge and dairy sludge) and biofertilizer (*Azotobacter chroococcum*) on the planting conditions of *Jatropha curcas* in metal contaminated soils. Results showed that the plants survival rate in heavy metal contaminated soil increased with addition of amendments. Treatment T6 (heavy metal contaminated soils + dairy sludge + biofertilizer) observed to be the best treatment for growth (height and biomass) as compared with the treatment T5 (heavy metal contaminated soils + biosludge + biofertilizer). In addition, organic amendments provided nutrients such as carbon, N, P and K to support plant growth and reduced the metal toxicity to plant. The present study showed that metal contaminated lands/soils could be suitably remediated by adapting appropriate measures. (Source - Kumar et al 2008, Bioresource Technology 99(6) 2078-2082)

Effect of liquid and cyst formulations of *Azospirillum* with inorganic nitrogen on the growth and yield of

rice - The nitrogen-fixing rhizobacterium *Azospirillum* lives in close association with plant roots, where it exerts beneficial effects on the plant growth and yield of many crops of agronomic importance. As carrier-based inoculants have a short shelf life and poor quality, new liquid and cyst formulations of inoculants have been developed and standardized for *Azospirillum*. In the present investigation, experiments were conducted to study the effect of liquid and cyst formulations of *Azospirillum*, combined with inorganic nitrogen, on the growth and yield of rice. Inoculation with the cyst formulation of *Azospirillum* enhanced the plant height, biomass and N uptake of the plants, the available nitrogen content of the soil and the yield of rice to the greatest extent when compared to carrier-based *Azospirillum*, followed by the liquid formulation. The results of the present study clearly indicated that the cyst and liquid formulations of *Azospirillum* could be used as bioinoculants more effectively than the carrier-based one. (Source Vendan and Thangaraju 2009 Acta Agronomica Hungarica Volume 57, Number (1) 57-65)

Improving nutrient uptake in wheat through cultivar specific interaction with *Azospirillum* -

Obtaining sufficient plant available nitrogen in organic dryland wheat cropping systems is difficult. This study was conducted to determine whether inoculation with *Azospirillum* could improve nitrogen uptake and increase crop yield, and whether there are differences among wheat cultivars in the ability to benefit from inoculation of these diazotrophic bacteria. Seed from twenty historic and modern wheat cultivars were either left untreated, or treated with a commercial inoculant of *Azospirillum*, and planted at two locations under certified organic

management. In one location with lower fertility, inoculation significantly increased yield and protein, and clear differences existed among individual cultivars in response to the inoculant. In another location with higher fertility, none of the cultivars responded as favorably to the inoculant, and yield in some cultivars was reduced. Plant breeders should be able to select for beneficial cultivar interactions with *Azospirillum* to increase wheat yield and protein levels. Additional research is needed to determine the impact of site-specific soil conditions on the effectiveness of *Azospirillum* in organic systems. (Source – Hoagland et al 2008, 2nd Conference of the International Society of Organic Agriculture Research ISOFAR, Modena, Italy, June 18-20, 2008)

A review of effects of biofertilizers on crop yield and quality - Effects of biofertilizer (except *rhizobium*) on crop yield and quality are reviewed, according to the literature in the past decade. Compared with controls of conventional fertilization, 98% trial of bio-fertilizer was documented to increase crop yield, among which 87.4% showed positive results of more than 5%, and 56.6% more than 10%. Average yield increase varies with different types of biofertilizer, in the order of mycorrhizal type>complex type>PGPR type>nitrogen-fixing type>photosynthetic bacteria type> K-solubilizing type, and their increasing rate were 22.3%, 21.2%, 16.5%, 14.7%, 13.6% and 12.2%, respectively, in terms of the kind of objective crops, biofertilizer application can be ranked as follows: cereals, oil crops, fiber crops, and tobacco, tea, herbs etc. Though the effects of biofertilizer on crop quality were not documented compared to crop yield, most such researches were conducted with cash crops. It might be considered that the biofertilizers play positive effect on product quality improvement. Generally, biofertilizer

application resulted in decrease of nitrate content for vegetables, hydroxybenzene for cotton seeds and erucic acid for rape seeds, and increase in sugar and vitamin C for vegetable and fruit, increase in length and intensity of cotton fiber and adding the proportion of first class ratio tobacco. The perspective is presented with respect to research, development and management of the biofertilizers. (Source TsingHua 2003, Journal of China Agricultural University)

***Azospirillum amazonense* inoculation effects on growth, yield and N₂ fixation of rice (*Oryza sativa* L) -**

Bacteria of the genus *Azospirillum* are considered to be plant-growth promoting bacteria (PGPR) and stimulate plant growth directly either by synthesising phyto-hormones or by promoting nutrition by the process of biological nitrogen fixation (BNF). The aim of this study was to isolate, characterise and evaluate auxin production and nitrogenase activity of *A. amazonense*. One hundred and ten isolates obtained from rice were characterised and grouped according to colony features. Forty-two isolates, confirmed as *A. amazonense* by the fluorescent in situ hybridization (FISH) technique, were tested for auxin production and nitrogenase activity in vitro. Subsequently plant growth promotion related to plant nutrition effect was evaluated, in vitro and in greenhouse experiments. The BNF contribution to plant growth was evaluated using the ¹⁵N isotope dilution technique. All *A. amazonense* strains tested produced indoles, but only 10% of them showed high production, above 1.33 μM mg protein⁻¹. The nitrogenase activity also was variable and only 9% of isolates showed high nitrogenase activity and the majority (54%) exhibited a low potential. The inoculation of selected strains in rice under gnotobiotic conditions reduced the growth of root and aerial part when

compared to the control, showing the negative effects of excess of phytohormone accumulation in the medium. However, in the greenhouse experiment, inoculation of strains of *A. amazonense* increased grain dry matter accumulation (7 to 11.6%), the number of panicles (3 to 18.6%) and nitrogen accumulation at grain maturation (3.5 to 18.5%). BNF contributions up to 27% were observed for *A. amazonense* Y2 (wild type strain). The data presented here is the first report that the PGPR effect of *A. amazonense* for rice plants grown under greenhouse conditions is mainly due the BNF contribution as measured by ^{15}N isotope dilution technique, in contrast to the hormonal effect observed with other *Azospirillum* species studied. (Source – Rodrigues et al 2008 Plant and Soil, 302, (1-2) 249-261)

Rhizobium oryzae* sp. nov., isolated from the wild rice *Oryza alta - During a study of endophytic nitrogen-fixing bacteria present in the wild rice species *Oryza alta*, eight novel isolates were obtained from surface-sterilized roots and classified in the genus *Rhizobium* on the basis of almost-complete 16S rRNA gene sequence analysis. These strains

can nodulate *Phaseolus vulgaris* and *Glycine max.* The highly similar protein patterns, DNA fingerprint patterns of insertion sequence-based PCR (IS-PCR) and DNA–DNA hybridizations showed that these novel isolates were members of the same species. The closest phylogenetic relatives of the representative strain Alt 505^T of the novel group were *Rhizobium etli* CFN 42^T and *Rhizobium indigoferae* CCBAU 71714^T, with 96.2 and 96.0 % 16S rRNA gene sequence similarity, respectively. Low DNA–DNA relatedness with the type strains of *R. etli*, *R. indigoferae*, *Rhizobium hainanense*, *Rhizobium mongolense* and *Rhizobium galegae* and differences in IS-PCR fingerprinting patterns, SDS-PAGE of proteins, antibiotic resistance, phenotypic tests and comparison of cellular fatty acids with *Rhizobium* species indicated that the novel group of isolates were distinct from previously described species. Based on these results, we propose to place them in a novel species, as *Rhizobium oryzae* sp. nov. The type strain is Alt 505^T (=LMG 24253^T =CGMCC 1.7048^T). (Source – Peng et al Int J Syst Evol Microbiol **58** (2008), 2158-2163)

Quality Control Legislations for Legume Inoculants (Rhizobium) in Canada and France

In Canada the legume inoculant (*Rhizobium*) standards are covered by the Fertilisers Act. Companies wanting to sell inoculants in Canada must submit registration to Agriculture Canada with data showing the product's efficacy. Agriculture Canada inspectors randomly collect and test about 150 inoculant samples each year from factory and sales outlets. Standards state that the inoculant must provide 10^3 – 10^5 rhizobia/seed, depending on seed size.

France also has legislation covering inoculants, although standards and procedures are slightly different. Inoculant products must be registered for sale in France. The product must have proof of efficacy and not be harmful to non-target crops, animals and humans and the environment. Thus, all inoculants are produced in sterile carriers. The inoculant must be able to deliver the equivalent of 1×10^6 rhizobia/soybean seed. Strains for particular legume species are also regulated. Inoculants are batch tested through INRA, Dijon, and certified if they pass the standard. They are retested if presented for sale in a second season.

Seminar, Conferences and Symposium News

Proceedings of the Group Meeting of All India Network Project on Biofertilizers held at YSPUHT, Solan, September 11-13, 2008

The group of the AINP on Biofertilizers was held at the Y.S. Parmar University of Horticulture and Technology from September 11-13, 2008. The inauguration of the programme was done by Hon. Minister for Health and Family welfare of H.P. Govt., Dr. Rajeev Bindal who outlined the importance of organic farming and biofertilizers for the state. The vice-Chancellor of YSPUHT, Solan, Dr. K.R. Dhiman drew attention to the opportunities available in the area in the hill state. Dr. P.D. Sharma ADG (Soils), ICAR mentioned the burden on account of the enormous fertilizer subsidy and stressed the need for using chemical fertilizers very judiciously. Dr. A. Subba Rao, Director, IISS highlighted the importance of integrated nutrient management. Dr. D.L.N.Rao, PC (AINP on BF) presented the progress of work in the project in the last few years and outlined the salient achievements which are listed in brief below:

Salient Achievements of AINP on BF

1. The first ever complete survey of Madhya Pradesh for soybean nodulation and inoculant usage completed. Best nodulation and yields observed wherever FYM and rhizobial inoculants were used together.
2. Molecular diversity of soybean rhizobia was assessed in Madhya Pradesh. High genetic diversity and evolution of native populations with time indicated the need for careful selection of competitive strains from the adapted populations.
3. Microbial Diversity of 'Diara' and 'Tal' lands of Bihar were assessed. Zero-tillage and intercropping promoted greater microbial numbers in IGP. One new N fixing *Azospirillum* spp. from maize producing black pigmentation identified.
4. New PSB- *Pseudomonas* isolated from Silent valley.
5. Mixed biofertilizers (BIOMIX) (N fixers, P solubilisers plus PGPR) promoted the growth of cereals, legumes and oilseeds better (10-25% increase) and saved 25% NP fertilizers in crops (rice, blackgram, chillis, cotton, soybean, pigeonpea, groundnut, pearl millet, wheat and mustard). Response of biofertilizers was better when used with chemical fertilizers.
6. Benefit due to biofertilizers in the inoculated soybean-wheat system was ~125 kg/ha/yr.
7. New co-culture medium formulated for growing mixed culture in same medium- of *Azospirillum lipoferum*, *Bacillus megaterium* and *Pseudomonas fluorescens*..
8. Liquid formulations were developed for *Rhizobium* (LM3), *Azospirillum* (LM2) and Phosphate solubilising bacterial (*Bacillus*) strains (LM3) with shelf life of one year without any contamination.
9. For spot assessment of quality of biofertilizers a quality assurance kit was developed using genetically marked strains. The technology has been filed for a patent.
10. Bioinoculants for vegetables in tribal areas of Orissa increased the yields by up to 20% for above ground and up

to 50 % for underground crops, saved 20-25% fertilizer cost, improved the nutrient use efficiency (12-36% for N, 18-28% for P, 9-15% for K and 16-18% for S).

11. The use of biofertilizers improved the quality of produce-for example anti-oxidants like lycopene increased by 13% and Vitamin C by 27 % in tomato, and curcumin content of turmeric increased by 10% in farmer field produce.
12. Biofertilizers for minor millets (Kodo millet and Kutki or little millet) and Niger in tribal areas of district Dindori in M.P gave yield increases of 5-11% over farmers practice where no fertilizers are being used. IPNS treatment resulted in substantial yield increase ranging from 100-230% over farmer's practice.
13. An endophytic bacteria from apple seedlings- *Bacillus megaterium* with multiple plant growth promoting activities could control white rot of apple significantly in Himachal.
14. A cost effective homestead method for round the year cultivation of *Azolla* has been developed at Jorhat. *Azolla* is grown in polythene lined pits and multiplies in 15-20 days. About 2-3 tonnes of *Azolla* can be supplied in each bigha of rice field with a supplementation of 4-8 kg N per crop per season (20-40 kg N/ha).
15. Mother cultures supplied to dozen companies. Consultancy given for setting up biofertilizer units.
16. In 'Front Line Demonstrations' application of biofertilizers gave additional groundnut pod yields 5-27% in Tamilnadu and 15-27% in Maharashtra; additional soybean seed yields of 5% in Madhya Pradesh and 30% in Maharashtra. In rice in Tamilnadu, 15-20% increase in yield with Azophos. In Haryana 5%

increase in grain yield and 6% in fodder yield was observed in pearl millet besides saving 25% N. In mustard 2.6- 9.0% increase in grain yield was obtained due to inoculation of mixed biofertilizers (increase of 0.5-1.5 q/ha.)

Session II:

The PI's of the 11 centres of the project presented their salient findings. This session was chaired by Dr. P.D. Sharma, ADG (Soils) and Dr. N.Trimurtulu (ANGRAU) acted as rapporteur.

Specific comments were made on the presentations by the PI's of the 11 centres of the project and action points identified.

The chairman, Dr. P.D.Sharma in his over-all comments complimented the scientists for the excellent work carried out by all the centres. He mentioned that all centres should have microbiological programmes and all the efficient organisms isolated should be pooled and characterized by using latest tools. These strains should be tested at national level to come with firm conclusions.

Session III:

In session III on 12th September 2008 chaired by Dr. A. Subba Rao with rapporteur Dr. Radha Prasanna, three invited presentations were made by eminent experts in the field of Biofertilizers. Dr. S.S. Dudeja, HAU, Hisar spoke on "*Rhizobium inoculation of pulse crops: current strategies and future needs*" and reviewed the work on Rhizobial inoculants, with special emphasis on Chick pea, The next lecture "*Integration of microbial inoculants in organic pulses production*" was delivered by Dr. Mohan Singh, IIPR, Kanpur who emphasized the need for inclusion of

organic farming practices in all cropping systems, especially pulses. Dr. A.K. Yadav, NCOF gave a very interesting lecture on aspects related to inclusion of Biofertilizers in the Fertilizer (Control) Order. A very interactive discussion took place on what parameters should be included in the specifications, and the problems in the presently included specifications for the different biofertilizer preparations using *Rhizobium*, *Azotobacter* and PSB. The final presentation in this session was by Dr. D.L.N. Rao, who provided an overview of the Biofertilizers /inoculants being developed in other countries of the world and presented slides showing the set up in Australia. He particularly stressed on the quality standards that need to be included in the Indian scenario, keeping in mind the specifications used in Australia, for improving the quality and thereby its widespread utilization by farmers.

Session IV:

In session IV chaired by Dr. T.K. Adhya, CRRI, with Dr. D. Balachandar, TNAU as rapporteur.

The technical programme for the next two years was discussed for all the centres. The various projects were grouped into 4 sub-heads and discussed. Microbial Diversity, Genetic diversity of rhizobia, Soil Genomics and Biofertilizer Diversification.

Overall recommendations

1. Continue the work on microbial diversity with greater vigour and special attention be paid to rain-fed dry lands, desert ecosystems, lands prone to periodic flooding and submergence, acid soils of east India and NEH region.
2. The work on genetic diversity of soybean rhizobia should be extended to other legume rhizobia viz., groundnut, blackgram, green gram, pigeonpea and chickpea.

3. Study the microbial diversity and soil health connection by assessment of the functioning of the genes involved in nitrogen transformations in soils.
4. Work on formulation and testing of mixed biofertilizers should be continued in IPNS mode and attention be paid to sugarcane, maize and other high nutrient removing crops.
5. The liquid inoculant formulations developed should be tested at other centers particularly under stress situations and also improved delivery systems may be devised and tested.
6. The diversification of biofertilizer researches be continued with attention to medicinal and aromatic plants, floriculture, forest tree species.
7. The demonstration on biofertilizers be continued with emphasis on transfer of the new technology developed during last five years like co-cultured formulations, new carrier based biofertilizers (incorporated with additives to improve shelf life), liquid biofertilizers, etc. Demonstrations of biofertilizers in tribal areas should be continued.

The work on impact analysis of biofertilizers done for soybean in central India, should be done at country level by developing linkages with user agencies, developmental agencies, line-departments, economists and policy makers.

Eighth European International conference on biological nitrogen fixation was held on 30th August to 3rd September 2008 in Ghent, Belgium.

This Conference was the 8th in a series of Conferences that brings together researchers with a passion for biological nitrogen fixation. With 305 participants coming from 34 countries it may

conclude that the community is alive and kicking! Going back to the goal of these meetings to strengthen European collaborations in the field, to foster scientific and technological cooperation between Europe and the rest of the world, particularly with developing countries and to offer a training ground for junior researchers it was agreed to pursue these lines and foster better coordination.

The final update of this website provides dedicated links to the pdf versions of the abstract books of the 8th European Conference on Nitrogen Fixation with Non-Legumes. Scrolling through these it is to announce that Hans-Martin Fischer and Xavier Perret have agreed to organise the 9th European Nitrogen Fixation Conference in 2010. The meeting will take place from 6 - 10 September, 2010 at the Centre International de Conférences Genève.

USDA-ARS National *Rhizobium* Germplasm Collection

The Agricultural Research Service (ARS) of the U.S. Department of Agriculture (USDA) maintains a collection of nitrogen-fixing legume symbionts with the following objectives to:

1. Provide, technical information about rhizobia, their preservation, and cultural and symbiotic characteristics.
2. Acquiring and preserving the nitrogen-fixing bacterial symbionts of leguminous plants with the goal of maintaining widest possible genetic diversity.
3. Maintaining quality control of new and existing germplasm by evaluation of microbiological purity and by examination of nodulation of the original trap host plant.
4. Distributing cultures to the public and private sectors
5. Developing or adapting techniques in molecular biology for the determination of genetic diversity of rhizobia, to investigate interactions with their host plants and to identify novel characteristics.
6. Acquiring, maintaining, evaluating quality, and distributing type strains for all the different taxa of nitrogen-fixing legume symbionts.
7. Participating in the UNESCO program.

Since 1975, the accessions have been maintained as suspension cultures in 20 percent glycerol stored at -70 °C, as well as in lyophilized form. Also, a separate set is stored in lyophilized form for disbursement in response to requests for cultures. Newly acquired cultures are checked for contamination upon their receipt and are verified as legume symbionts by plant testing for nodulation on the original host of isolation. Soil samples are collected from centers of origin of legume hosts for the isolation and acquisition of additional biodiversity.

The germplasm resource maintains all the type strains of the genera and species of legume symbionts and is the source for the type strains recognized by the International Subcommittee on the Taxonomy of *Agrobacterium* and *Rhizobium*. Cultures are distributed free of charge to recognized institutions and scientists at home and abroad.

Address - U.S. Department of Agriculture, Agricultural Research Service, Soybean and Alfalfa Research Laboratory, BARC-West, 10300 Baltimore Boulevard, Building 011, Room 19-9, Beltsville, MD 20705

Books Review

Biofertilisers in Action : By Ivan R Kennedy and Abu T M A Choudhury
Publication no: 02-086 Pages: 144

Price - \$35.00 - This publication is a product of the 8th International Symposium on Nitrogen Fixation with Non-Legumes, held at the University of Sydney in December 2000. The theme of Biofertilisers in Action was adopted for the Symposium and this book comprises a set of invited papers selected as most relevant to this theme. This is a forward-looking book related to the future application of the plant growth-promoting rhizobacteria (PGPR) to provide help more sustainable organically-based agriculture. This book is a genuine attempt to set the stage for this development. If justified by subsequent field trials and economic and environmental advantages, the area has a major potential to influence agriculture.

Biological Nitrogen Fixation: Towards Poverty Alleviation through Sustainable Agriculture.

Proceeding of the 15th International Nitrogen Fixation Congress and the 12th International Conference of the African Association for Biological Nitrogen Fixation .Series: Current Plant Science and Biotechnology in Agriculture, Vol.42 Dakora, F.D.; Chimpango, S.B.M.; Valentine, A.J.; Elmerich,C.; Newton, W.E.(Eds.) ISBN: 978-1-4020-8251-1 Online Version available. This volume

covers recent developments in both fundamental and applied research in biological nitrogen fixation. It emphasizes the application of biological nitrogen fixation for sustainable agriculture, which should lead to poverty alleviation, environmental protection, and good agricultural practices generally. The roles of and advances in, plant breeding, plant molecular biology, nodule physiology, and symbiotic and associative interactions between plants and microbes in sustaining agricultural productivity and soil fertility are described. The evolution of symbioses and nitrogen fixation are also covered in this volume. To ensure high agricultural productivity, while protecting the environment (both soil and water resources), requires plant cultivars that also respond to beneficial microbes. The volume, therefore, describes the physiology and genomics of nitrogen-fixing bacteria together with the biochemistry and molecular genetics of the nitrogenase enzyme that actually fixes atmospheric nitrogen to a usable form. This volume, which covers the most recent data on the role of nitrogen fixation in agriculture and forestry and on the biology of both plants and nitrogen-fixing microbes, is intended to serve as a useful reference for students and researchers, both in the laboratory (academic and commercial) and in the field.