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Contents

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From Editor's Desk

Dear Readers,

Biofertilisers or "Microbial Inoculants" have come a long way during the last 30 years in the country. India is now among the largest biofertiliser producers and consumers in the world. But in spite of this spectacular growth, the quality still continues to be a cause of concern. Every year the concerns are being raised in different Seminars and Conferences, but the scenario has not changed much. Still large numbers of producers are using the age old un-sterile technology and are delivering contaminated product with unknown status of total viable count. The major problem is not in their storage and transport (as often stated), but lies mainly in the production technology itself, which need to be upgraded first. Once the short comings are overcome at production front, I think other problems can be sorted out without much difficulty.

For quality determinants, Bureau of Indian Standard has issued necessary specifications in respect of four important biofertiliser organisms. But interestingly less than 5% of the firms have actually obtained BIS certification. This is the clear evidence of poor status of quality. It is now high time that, instead of concentrating on establishment of more and more production units, we must concentrate on technological up-gradation of existing units and ask all production units to obtain BIS certification. The unsterile production system need to be dispensed with immediately and a new system of sterile production should be brought in with proper automation. Liquid inoculants can also play an important role in this change over and can lead to revolution in the scenario.

Limited potential of free-living biofertiliser organisms in nutrient mobilization, (other then rhizobia) has also attracted the attention of scientists. Increasing attention is now being directed in search of endophytic or associative systems. In this search, although, various new microorganisms have been identified from nature and their potential are being evaluated, but they are yet to be commercialized. Vesicular-arbuscular mycorrhizal (VAM) inoculants are also awaiting mass scale production and utilization.

Cyanobacterial (or BGA) inoculants were although, taken up by the State Agriculture Departments on large scale, but the gaps in technology has resulted in failure. Scientists have now come up with improved technology and improved inoculant quality, which holds much better promise for their mass scale commercialization. All these sectors need to be properly tapped and exploited for the increased role of biofertilisers in the emerging prospects of organic farming. I invite all the scientist, industrialists and technologists to join the hands and work together for the better future of biofertilisers.

A.K. Yadav
Editor

Burkholderia sp. – Another Promising Endophytic Diazotroph for Sugarcane

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सारांश

गन्ने की विभिन्न भारतीय प्रजातियों में नत्रजन स्थिरीकारक अंतःजीवी जीवाणुओं की खोज के दौरान तामिलनाडु राज्य में उगाई जाने वाली कुछ प्रजातियों की जडों व अन्य उपरी भागों में अंतःजीवी जीवाणुओं के एक नये समूह का पता चला। ये जीवाणु गन्ने में पाये जाने वाले अन्य नत्रजन स्थिरीकारक जीवाणुओं से बिलकुल अलग थे और कॉलोनी आकारिकी तथा फैटी अम्ल संरचना में बुरखोलडेरिया वियतनामियेन्सिस (TVV75^T) से मिलते जुलते थे। गन्ने के पौधों पर इन जीवाणुओं के लाभदायी प्रभाव जाँचने के लिये इन्हें अकेले व अन्य लाभदायी जीवाणुओं के साथ गमले व प्रक्षेत्र परीक्षणों में गन्ने की CO-86032 प्रजाति के साथ जाँचा गया। गमला परीक्षणों में इन नये जीवाणुओं को अकेले व ग्लूकोनएसिटोबैक्टर डाइएजोट्रोफिकस (PA 15^T) के साथ जाँचा गया तथा प्रक्षेत्र परीक्षण में इन जीवाणुओं को अकेले व ग्लूकोनएसिटोबैक्टर डाइएजोट्रोफिकस तथा हर्बास्पिरिलम सिरोपिडेसी (LMG6513^T) के साथ जाँचा गया। सभी परीक्षणों में अकेले जीवाणुओं के नत्रजन की कम मात्रा के साथ प्रयोग से सर्वाधिक उत्पादन वृद्धि प्राप्त हुई। तीनों जीवाणुओं के बीच बुरखोलडेरिया का प्रयोग अन्य दो के मुकाबले अधिक प्रभावी था। इन परीक्षणों द्वारा बुरखोलडेरिया जीवाणु की गन्ने की फसल के लिये एक नये जैव उर्वरक रुप में उपयोगिता सिद्ध होती है।

Introduction

Sugarcane crop obtains 20–60% of its N-requirements from the associative microsymbionts (Boddey *et al* 2001; Oliveira *et al* 2002). *Gluconacetobacter diazotrophicus* has been shown to contribute significant amounts of N-need of sugarcane (Sevilla *et al* 2001). *Herbaspirillum* spp. exists within sugarcane as endophyte and their promotion of plant growth has been reported (Muthukumarasamy *et al* 1999). The genus *Burkholderia* was not recognized as diazotrophic for a long time. Presently, it includes 30 known species (Coenye and Vandamme 2003) with *B. cepacia* as the type species. *B. vietnamiensis* was the first known nitrogen fixing species reported from rice plant in Vietnam (Gillis *et al* 1995). Later, presence of diazotrophic *Burkholderia* sp. in sugarcane was reported by Baldani (1996). Species of environmental origin of this genus have been recorded in the last few years (Viallard *et al* 1998; Achouak *et al* 1999; Yabuuchi *et al* 2000; Zhang *et al* 2000; Brämer *et al* 2001 and Goris *et al* 2003). Recent report suggests that many more species of this genus actually include diazotrophic strains (Estrada *et al* 2001 and 2002). More recently, *B. tropica* (Reis *et al* 2004) and *B. unamae* (Caballero-Mellado *et al* 2004) have been described as new diazotrophic species. Our recent survey on nitrogen-fixers associated with sugarcane varieties in Tamilnadu showed the presence of endophytic *Burkholderia* spp. The potential of *Burkholderia* sp. as a diazotrophic bio-inoculant for sugarcane was examined in micro-propagated sugarcane

plantlets. Both pot and field trials conducted at Chengalpattu, Tamilnadu state revealed its efficiency in N-saving as well as growth promotion of sugarcane crop.

Materials and Methods

Plant samples - Triplicate samples of root, stem from sugarcane varieties of Co 86032, Co 86027 and Co 8021 grown under field conditions were collected from Kavithandalam and Cheyyar of Kanchipuram district; Tirupattur and Ambur of Vellore district in Tamilnadu state.

Enrichment culture media - N-free LGI M (Estrada *et al* 2002) semi solid medium was used for enrichment. Modified PCAT medium (Estrada *et al* 2001 and 2002) was used (omitting tryptamine and chlorothalanil and adding bromothymol blue) for isolation of *Burkholderia* species, as this medium is considered selective for the genus. BMGM medium supplemented with three carbon sources such as glucose (0.2%), malic acid (0.2%) and mannitol (0.1%) was used for enumeration studies (Estrada *et al* 2001).

Isolation and enumeration - Root and stem samples of the above three sugarcane varieties were thoroughly washed first with running water to remove the soil followed byMgSol (10 mM $MgSo_4$ $7H_2O$) (Estrada *et al* 2001) and vortexed for 3 min. 1 g of roots were washed with sodium hypochlorite (4%) followed by a sterile water wash thrice. The roots were rolled on to Luria-Bertani agar to verify root surface sterilization and then macerated and the suspension was used for serial dilution. 10 g of stem samples cut into pieces of 5 – 6 cm were surface sterilized with 95% ethanol for 10 min, washed 5 times in sterile water and homogenized using a pestle and mortar in 100 ml MgSol. The homogenized samples were serially diluted. 0.1 ml of each root and stem samples were inoculated into vials (triplicate) containing 5 ml of N-free semi-solid LGI M with an initial pH of 6.0. After 72–96 h of incubation, vials were assayed for acetylene reduction activity (ARA, Muthukumarasamy *et al* 1999). ARA positive vials with white yellowish sub-surface pellicle at a depth of 1–4 mm below the surface were replicated again into LGI M medium, incubated for 96h and then streaked on to modified PCAT agar plates (pH 5.7). Both PCAT (Pallud *et al* 2001) and BMGM (Estrada *et al* 2001) were incubated at 29°C for a week and observed for colony morphology. Fresh N-free semi-solid BMGM vials inoculated with 100µl aliquots from diluted samples were incubated for 72 h at 29°C for MPN counts using McCrady table.

Characterization of *Burkholderia* spp. - Microscopic observations were made using a Phase Contrast Fluorescent Microscope (Olympus BX 41). Oxidase and catalase tests were determined using commercially available discs (Himedia). Growth of the isolates on different carbon sources was tested using BMGM in which carbon source was replaced by individual carbon substrates such as D-glucose, sucrose, L-raffinose, meso-inositol, L-rhamnose, mannitol, cellobiose, meso-erythritol, succinic acid, oxalic acid, tartaric acid, malic acid and citric acid. 5 g/l of individual carbon source and ammonium chloride (500 mg/l) were used in this study. Growth on amino acids like L-cysteine, L-glutamine, L-proline, L-tryptophan, L-leucine, L-threonine, L-histidine, L-lycine, L-tyrosine and L-valine was studied in the presence of sorbitol as a carbon source.

Nitrogenase activity - ARA was performed in 10 ml vials containing 5 ml of semisolid BMGM inoculated with single colonies and kept at 30°C for 48 h. After incubation, 10% acetylene (v/v) was injected into the vials, sealed with suba-seal rubber stoppers. These

tubes were incubated for 1 h. Ethylene production was measured using a flame ionization Gas Chromatograph (Systronics) with Poropak Q column. Uninoculated vials were used as negative controls.

Fatty acid analysis - MIDI-FAME technique was used to determine the cellular fatty acids from isolate TNCSF 43B and type strain (TVV75^T). Isolates were grown overnight on trypticase soy agar plates. One loopful of live wet cells were harvested and transferred to screw cap culture tubes. One ml of saponification reagent was added to each tube and tightly sealed with a teflon-lined screw cap and vortexed for 5-10 seconds. The sample tubes were then placed in water bath at 100 ± 2°C for 5 min. The tubes were removed from the boiling water bath, cooled slightly, vortexed for 10 sec and incubated in a water bath for additional 25 min. After a total of 30 min of saponification in the water bath, the samples were placed under tap water. To each tube 2.0 ml of methylation reagent was added, tightly capped and vortexed for 10 sec. The tubes were placed in the water bath at 80±1°C for 10 min. Then the samples were cooled at room temperature by placing the tubes in a tray of cold tap water. Then, 1.25 ml of extraction reagent was added into the tubes. The tubes were placed on a laboratory rotator for 10 min. After the spin, the bottom phase was removed using a micro pipette. Finally 3.0 ml of base reagent was added and this was placed again for 5 min in a centrifuge. The upper solvent phase was removed and transferred to vials for fatty acid analysis.

Estimation of IAA - IAA production of these bacterial isolates were determined by growing the cultures in CC medium with sucrose (5g l^{-1}), mannitol 95 g l^{-1}) and ammonium chloride (1 g l^{-1}). Tryptophan (100 mg l^{-1}) was added as the precursor and 7 d old cells were harvested and pelleted (at 10000 rpm for 15 min.). pH of the supernatant was adjusted to 2.8 with HCl and extracted three times with an equal volume of ethyl acetate (Tien *et al* 1979) evaporated to dryness and re-suspended in 1ml of ethanol for analyzing with HPLC (Shimadzu SPD 10A, LC10AD – using U.V. detector and C–18 column). Pure IAA was used as standard for identification and quantification. Methanol: Acetic acid: water (30:1:70 v/v/v) was used as a mobile phase at the rate of 1.2 ml min^{-1}. (Rasul *et al* 1998).

PCR-Amplification of the *nif* genes - The *nif*D sequence of amplification primers was *nif* 'DF' 5-ATSGARTWCAACTTCTTCGG -3 for 883f and *nif* DR –5-ARTCCC AIGAGTGCATYTGICGGAA-3 for 1337 R (where I represents inosine, R represents A or G, S represents C or G, W represents A or T and Y represents C or T) (Ueda *et al* 1995a). Amplification reactions were performed in a total volume of 25 µl. The reaction mixture contained; 2.5 µl 10X PCR buffer, 2.5 µl of 2mM each of dATP, dCTP, dTTT and dGTP; 3 µl of each forward and reverse primer (30 ng), one µl of template DNA (10 ng) and 0.3 µl of (3 unit/µl) enzyme; and made up to a final volume of 25 µl using milli-Q water. The amplification reaction was performed for denaturation at 95ºC for 3 min, 52ºC for 1 min, and 72ºC for 1 min, followed by 30 cycles of 95ºC for 1 min, 54ºC for 30 sec and 72ºC for 1 min, with a final extension at 72ºC for 10 min. Amplification products were analyzed on 1.5 agarose gel in 1X TBE buffer. For *nif*H gene, the degenerated primers were: 5-GCIWTYTAYGGIAARG GIGG –3 for 19 F and 5-AAICCRCCRCAIACIACRTC-3 for 407 R (where I represents inosine, R represents A or G, S represents C or G, W represents A or T and Y represents C or T as described by Ueda *et al* (1995b). The reaction mixture contained; one µl of template DNA (10 ng), and 2.5 µl 10X PCR

buffer, 2.5 µl of 2 mM each dATP, dCTP, dTTT and dGTP, 3 µl of each forward and reverse primer (30 ng), and 0.3 µl of (3 units/µl) enzyme and made up to a final volume of 25 µl using milli-Q water. DNA amplification was performed with the following conditions; initial denaturation was done for 4 min at 94ºC, 30 sec at 94ºC, 1 min at 50ºC and 30 sec at 72ºC for 40 cycles with a final extension at 72ºC for 10 min. Amplification products were analysed on 1.5 % agarose gel in 1XTBE buffer.

Micro-propagation of sugarcane- Micro-propagated sugarcane seedlings were produced from apical meristems of the sugarcane variety Co 86032, following the method described by Sreenivasan and Sreenivasan (1984). Modified MS liquid and solid media were used to produce well-developed seedlings in about 80 days following axenic growth conditions (Murashige and Skoog, 1962). With suitable concentrations of growth hormones in the basal medium, initiation of callus and multiplication of aerial shoots and roots were performed. The seedlings with uniform size after selection were transferred to tubes having minimal salt concentrated MS medium (1/10) (James *et al* 1994). Three days after incubation only those tubes with no apparent contamination were used for inoculation of diazotrophic cells of *Burkholderia* sp., *G. diazotrophicus* and *H. seropedicae*. A 0.1 ml of bacterial suspension containing 10^7 to 10^8 cells ml^{-1} was used for inoculating into micro-propagated plants. Plants were maintained at 27°C with an illumination of 70 µmol m^{-2} s^{-2} for 16 h d^{-1}. After 7 d of inoculation, the plants were kept for primary hardening, maintained under 50% light intensity until the plants reached 75 - 100 mm. These were further used for pot and field experiments.

Pot Experiment - 50 l plastic containers filled with 40 kg of thoroughly mixed upper layer of a farm clay soil (pH 7.5., EC 0.13 m mhos/cm; available N 114.5 kg ha^{-1}; available P 28.6 kg ha^{-1}; available K 270.85 kg ha^{-1}; organic carbon 0.32%) was used for the pot trial. There were 9 treatments and 6 replicates. Treatments include *Burkholderia* strain (TNCSF 43B), *G. diazotrophicus* (ATCC 49037), a combination of two at two levels of N-fertilisers – recommended rate (280 kg N ha^{-1}) and reduced rate (140 kg N ha^{-1}). Unionoculated controls with 2 levels of N (280 and 140 kg N ha-^{1}) and without N were also maintained.

Field experiment - Field experiment was conducted on clay soils at Kavithandalam area, Chengalpattu, Tamilnadu state using micropropagated sugarcane seedlings of the variety Co 86032. Each plot was of 6 m^2 with total experimental area of 144 m^2. The trial had 6 treatments and 4 replicates in Randomized Block Design (RBD). *H. seropedicae* (LMG 6513^T) another diazotrophic strain along with two strains used in pot experiment were included for the treatments in the field trial. Fertiliser N was reduced to half (140 kg N ha^{-1}) of the recommended rate (280 kg N ha^{-1}). Seedlings inoculated with autoclaved bacterial cells were used as controls. Phosphorous and potassium were applied at 65 and 115 kg ha^{-1} for all treatments.

Results and Discussion

Isolation of *Burkholderia* strains from roots and shoots of sugarcane varieties of Tamilnadu state is reported in this study (Table 1). PCAT medium was considered as selective for *Burkholderia* strains. Predominant colonies on PCAT agar were opaque, white or whitish, round, shining with entire margins. On LGI M agar plates, colonies were round, yellowish, mucoid, smooth and translucent with entire margins.

On the basis of morphological and fundamental biochemical tests, the isolates were identified as *Burkholderia* spp. AR assay revealed that pH 5.0-5.5 was the optimum level for high nitrogenase activity (Table 2). The detection of *nif*D and *nif*H on the selected strains confirmed their diazotrophic nature. These isolates were Gram negative, oxidase and catalase positive and utilized sugars like (0.5%) glucose, sucrose, sorbitol, meso-inositol, mannose, glycerol, L-rhamnose, fructose, mannitol, cellobiose, xylose and N-acetyl glucosamine, but they did not use starch and maltose. They also utilized organic acids like azelaic, malic succinic, valeric, fumaric, hippuric, tartaric and citric acids but failed to use adipic, malonic, ά-ketoglutaric and oxalic acids. All the isolates used amino acids such as L-threonine, cysteine, glutamine, proline, tryptphan, leusine, lysine, histidine, tyrosine and valine as N sources.

Cellular fatty acid analysis

The cellular fatty acid analysis of the isolates TNCSF43B and type strain (*B. vietnamiensis*) showed that 14.0 and 16.0 types were predominant. The type strain had 14.62% fatty acid of 16.0 –OH while TNCSF 43B had 22.42% (Table 3). This isolate also had a lesser content of 16.0 2OH, 16.1 2OH and 18.0 type fatty acids than the type strain. The TNCSF 43B had a higher quantity of 17.0 cyclo and 18.1 2OH fatty acids than the type strain. The presence of 16:0 3-OH is the characteristic feature of *Burkholderia* genus and the result confirmed that the isolates under study were phylogenetically coming under the genus *Burkholderia* as suggested (Viallard *et al* 1988).

Table 1. Origin of diazotrophic *Burkholderia* spp. obtained from roots and stems of sugarcane grown in various parts of Tamilnadu state, India.

Strain code	Variety used for isolation	Plant part	Location
TNCSF 87B	Co 86032	Surface sterilized root	Kavithandalam, Kanchipuram District
TNCSF 43B	Co 86032	Surface sterilized root	Tirupathur, Tirupathur District
TNCSF 44B	Co 86027	Surface sterilized stem	Cheyyar, Kanchipuram District
TNCSF 32B	Co 8021	Surface sterilized stem	Ambur, Vellore District

Table 2. Acetylene Reduction Assay by representative isolates at different pH levels on N-free semisolid BMGM medium (n mole of C_2H_4 per tube, 10 ml medium after 72 h of growth)

Isolate code	pH levels					
	4.0	4.5	5.0	5.5	6.0	6.5
TNCSF 87B	40.42	346.46	525.88	379.11	216.98	115.12
TNCSF 43B	30.38	278.19	3118.69	4262.58	1351.39	782.38
TNCSF 44B	144.17	1200.38	2413.11	998.12	615.91	341.18
TNCSF 32B	34.69	270.12	703.72	282.58	80.96	64.42
$TVV75^T$	6.2	344.15	1245.92	575.43	241.17	158.38

$TVV75^T$ - Type strain of *B. vietnamiensis*

Values represent nano moles of C_2H_4 per tube and means of three replicate cultures. When inconsistent ARA was observed, five replicates were done in independent experiments.

Table 3. Fatty acid composition of representative isolates

Name of the fatty acid	TVV75^T	TNCSF 43B
10.0	0.47	-
13:1	-	0.50
14.0	6.57	5.82
16.0	14.62	22.42
16.0 2 OH	1.86	1.56
16.0 3 OH	6.20	5.96
16.1 2 OH	3.03	2.62
17:0	-	1.24
17.0 CYCLO	3.00	19.0
18.0	1.67	0.72
18.1 2 OH	2.16	2.84
19.0 CYCLO W8C	0.67	5.5
19.0 Methyl	-	-
20.0	-	-
20.0 ISO	-	-
20.1 W9T	-	-
Sum in feature-3	13.96	9.76
Sum in feature-4	25.71	10.41
Sum in feature-7	20.09	11.61

Summed Feature 3 - unknown 10.928
Summed Feature 4 - 15 : 0 ISO 2OH /16:1 w7c
Summed Feature 7 - 18 : 1 w9c / w12t / w7c
TVV75^T - Type strain of *B. vietnamiensis*

Production of IAA - Test isolates of *Burkholderia* produced IAA in the range of 167 to 364 mg l^{-1} when supplemented with tryptophan as the precursor and in the range of 13 to 28 mg l^{-1} without the precursor. *Burkholderia* spp. were reported to produce efficient siderophores and IAA (Tran Van *et al* 1994).

Inoculation effects on pot and field experiments - Pot trial had the treatments of *G. diazotrophicus*, a local root endophytic *Burkholderia* species and a combination of both. MPN counts of the inoculated species showed the colonizing ability of these diazotrophic strains in sugarcane plants. When root colonization of inoculated strains was examined, it was observed that numbers of *Burkholderia* sp. remained stable for 180 d with a density of 10^5 cfu per g (fresh root tissues) whereas populations of *G. diazotrophicus* gradually declined after 120 d (Table 4). In the dual inoculation also, numbers of *Burkholderia* sp. remained same but *G. diazotrophicus* declined. Uninoculated plants showed absence of N_2 fixing bacteria at the beginning but later appeared progressively, except in the full N dose treatment. In field trial, the numbers of *Burkholderia* sp. and *H. seropedicae* remained same till the harvest as reported earlier (Muthukumarasamy *et al* 1999a: Reis Jr *et al* 2000). Population of *G. diazotrophicus* declined gradually after 90 d of application when applied individually or with *Burkholderia* (Table 6). The decline in the numbers of *G. diazotrophicus* in relation to the plant age was already reported in Mexican sugarcane varieties (Munoz-Rojas and Caballero-Mellado, 2003). Of all the introduced bacterial treatments, inoculation

of *Burkholderia* sp. with 50% N (140 kg N ha^{-1}) showed highest biomass both in pot and field experiments. The effect of combination showed varying results (Table 5 and Table 6).

Table 4. Numbers of diazotrophic bacteria in LGIP and PCAT medium from the roots of micro-propagated sugarcane variety Co. 86032 grown in pots with 65 & 115 Kg ha^{-1} of P & K respectively.

Treatments	Root bacterial populations (X 10^4 g^{-1} fresh wt) days after inoculation				
	60^{th}	90^{th}	120^{th}	150^{th}	180^{th}
[a]280Kg N ha^{-1}	0	0	0	0	0
[a] 140 Kg N ha^{-1}	0	0	0	0.11	0.21
[a] 0. Kg N ha^{-1}	0.01	0.11	0.21	0.40	0.40
140. Kg N ha^{-1} + *Gd*	11.5	11.5	11.5	7.35	4.35
140. Kg N ha^{-1} + *Bsp*	11.5	7.35	11.5	11.5	11.5
140. Kg N ha^{-1} + *Gd*+*Bsp*	11.5	11.5	11.5	4.35	4.35

Gd – G. diazotrophicus (PAL 5^T), *Bsp – Burkholderia* sp. (TNCSF 43B)
Arithmetic zero represents value beyond detection limit $<10^4$
[a] Seedlings inoculated with autoclaved cells

Table 5. Effect of inoculation of *G. diazotrophicus* (*Gd*) and *Burkholderia* sp. (*Bsp*)on leaf N content and biomass of micro-propagated sugarcane variety Co. 86032 grown in pots for 180 d with 65 & 115 Kg ha^{-1} P & K respectively.

Treatments	Biomass (g^{-1}) [a]			Leaf N[b]
	Root	Shoot	Total	(mg g^{-1} dry wt.)
280 Kg N ha^{-1}	190.83 ab	1798.33 a	1977.50 a	6.87 a
140 Kg N ha^{-1}	165.83 abc	1685.33 a	1851.66 a	5.77 bc
0 Kg N ha^{-1}	55.83 d	821.66 c	877.50 c	3.69 d
140 Kg N ha^{-1} + *Gd*	183.33 ab	1744.16 a	1928.33 a	6.10 b
140 Kg N ha^{-1} +*Bsp*	202.50 a	1779.16 a	1981.66 a	6.08 b
140 Kg N ha^{-1}+*Gd* + *Bsp*	140.00 abc	1354.16 ab	1494.16 ab	6.03 b
F-value	9.89	12.23	12.44	22.70
Coefficient of variation (%)	30.47	40.95	29.86	14.84

Different alphabet between treatments differing at 5% level (Tukey test)
[a] Means of six replicates, [b] Leaf N (Mean six months)
For other explanations, see Table 4

Table 6. Numbers of diazotrophic bacteria in LGIP, PCAT and JNFB medium in micro-propagated sugarcane variety Co. 86032 grown in the field with 65 & 115 Kg ha^{-1} of P & K respectively

Treatments	Bacterial populations (X 10^4 g^{-1} fresh wt.)[a], days after planting					
	30^{th}	60^{th}	90^{th}	120^{th}	150^{th}	180^{th}
280 Kg N ha^{-1}	0	0	0	0	0	0
140. Kg N ha^{-1} *	2.12	2.12	4.09	4.09	4.59	4.59
140. Kg N ha^{-1} + *Gd*	11.5	11.5	7.35	7.35	4.09	2.12
140. Kg N ha^{-1} +*Hs*	11.5	11.5	11.5	11.5	11.5	11.5
140. Kg N ha^{-1}+*Bsp*	11.5	11.5	11.5	11.5	11.5	11.5
140. Kg N ha^{-1} + *Gd* + *Hs* + *Bsp*	11.5	11.5	7.35	4.59	2.12	1.15
Hs	11.5	11.5	11.5	11.5	11.5	11.5
Bsp	11.5	11.5	11.5	11.5	11.5	7.35

Arithmetic zero represents cell numbers below 10^4 of *G. diazotrophicus* and *Herbaspirillum* sp. , [a] – means of two independent estimates
Hs – Herbaspirillum sp. (LMG 6513^T)

*- Numbers were (*Spirillum* spp. and *G. diazotrophicus*) less than 10^4 cells g^{-1} fresh wt.
For other explanations, see Table 4

Table 7. Effects of different diazotrophs on leaf N content and total biomass in variety Co 86032 grown in the field for 12 months with 65 and 115 kg ha^{-1} of P and K

Treatments	N Content (mg g^{-1})[a], days after planting						Total Biomass (tons / ha^{-1})
	60th	120th	180th	240th	300th	Mean	
280 kg N ha^{-1}(T_1)	2.45 c	4.37 a	5.00 a	5.60 a	4.37 a	4.35 b	163.12 c
140 kg N ha^{-1} (T_2)	2.45	1.64 b	3.25 b	4.27 cd	4.13 a	3.14 d	158.87 d
T_2 + *G d*	4.02 b	2.03 b	4.62 a	4.69 bc	4.30 a	3.93 c	185.52 b
T_2+ *Hs*	3.04 c	1.96 b	4.62 b	4.20 cd	4.48 a	3.66 c	178.75bc
T_2+ *Bsp*	4.13 b	1.92 b	3.11 a	4.34 d	4.37 a	3.57 c	191.00 a
T_2+ *Gd + Hs + Bsp*	10.22 a	2.24 b	4.79 a	4.97 b	4.44 a	5.33 a	181.45 b
F-value	233.61	50.65	12.36	25.88	0.22	15.22	16.31
Coefficient of variation (%)	63.24	40.92	20.75	11.32	11.03	15.28	1.38

Different alphabet between treatments differing at 5% level (Tukey test)
[a] Monthly data were means of six replicates
For other explanations, see Tables 5 and 6

When *Burkholderia* sp. was inoculated with *G. diazotrophicus* in the pot trial, the total biomass was reduced correlating with reduced leaf N content. The increased yields over the control were 13.7, 9.2, 17.09 and 10% for *G. diazotrophicus*, *H. seropedicae*, *Burkholderia* sp. and for the mixed inoculation respectively in the field trial (Table 7). Earlier Sevilla *et al* (2001) reported that diazotroph (*G. diazotrophicus*) was capable of fixing N_2 inside sugarcane plants and could promote the sugarcane plant growth considerably. The low numbers of *Burkholderia* sp. and *G. diazotrophicus* (~ 10^5 cells g^{-1} fresh wt) inside sugarcane plants observed in the present study reflected the earlier observations of Munoz-Rojas and Caballero-Mellado *et al* (2003). These authors observed a positive growth promoting effect on sugarcane plants, though *G. diazotrophicus* numbers found inside the sugarcane plants was less and they further suggested the growth promotion was possible when there is an appropriate interaction between sugarcane variety and bacterial genotype as it was observed in the present study. It is revealed that inoculated plants yielded more than uninoculated plants. Perhaps the growth promotion of inoculated plants may be due to the production of growth hormones like IAA (Fuentez-Ramirez *et al* 1993) by the inoculated bacteria apart from N contribution as suggested (Munoz-Rojas and Caballero-Mellado 2003; Tran Van *et al* 2000).

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National Biofertiliser Development Centre

is now

National Centre of Organic Farming

The National Project on Use and Development of Biofertilisers, under which National Biofertiliser Development Centre, Ghaziabad and its six Regional Biofertiliser Development Centres at Bangalore, Bhubaneshwar, Hisar, Imphal, Jabalpur and Nagpur were established, was launched during the year 1987-88. With the intensive efforts and initiatives taken up under this project, the production and consumption of different biofertilisers have increased from a mere 900MT (in1989-90) to well above 12,000MT (in 2003-04). Total numbers of production units in the country have also increased from just 30 to more than 220 over this period. With the increasing role of private sector and large scale availability of different biofertilisers, the Government of India during 2001-02 decided to withdraw the production activity from its National and Regional centres and proposed to use the set up for the promotion of emerging discipline of organic farming.

With effect from 01.10.2004, the National Project on Use and Development of Biofertilisers has been merged with the newly launched National Project of Organic Farming. All essential activities relating to biofertilisers such as maintenance and supply of strains and mother cultures, quality control of biofertilisers, information dissemination, new biofertiliser development and evaluation etc will continue to be looked after, under this new set up.

Under this new project National and its six Regional Biofertiliser Development Centres have been renamed as National Centre of Organic Farming and Regional Centre of Organic Farming.

Cyanobacteria (BGA) Biofertiliser Technology for Orissa State

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सारांश

धान की फसल में नील हरित शैवाल जैव उर्वरक की उपयोगिता सर्वविदित है परंतु इस जैव उर्वरक की उत्पादन प्रक्रिया में कुछ कठिनाइयों के कारण इस का प्रयोग अधिक नहीं हो पा रहा है। इन कठिनाइयों को दूर करने व अच्छी गुणवत्ता के जैव उर्वरक का उत्पादन सुनिश्चित करने के प्रयास किये गये और उडिसा राज्य की स्थानीय परिस्थितियों के अनुकूल एक उन्नत प्रक्रिया विकसित की गई जिसका विवरण इस लेख में दियाा जा रहा है। इस प्रक्रियाा में ८ विशिष्ट प्रजातियों का चयन कर उन्हें पॉलिहाउस के नीचे बने सीमेंट की नाँदों में अलग अलग उगााया जाता है तथा वाहक के साथ मिलाकर शुष्क जैव उर्वरक का निर्माण किया जाता है। तकनीक के प्रचार प्रसार के लिये एक ग्रामीण उत्पादन इकाई की भी स्थापना की गई है जहाँ प्रगतिशील किसानों को पूरी प्रक्रिया की जानकारी दी जाती है इस तकनीक के प्रयोग से ०.१ एकड जमीन पर लगभग ५०,००० रु के निवेश से एक छोटी उत्पादन इकाई की शुरुआत की जा सकती है जिससे प्रतिमाह ५,००० रु की आमदनी हो सकती है। एक एकड क्षेत्र में लगभग २५ लाख रु के निवेश से एक बडी इकाई की स्थापना संभव है जिससे प्रति माह लगभग २० से २५ हजार रु की शुद्ध आमदनी प्राप्त की जा सकती है।

Introduction

Agronomic potential of BGA has long been recognized as a promising source of nitrogen for rice. Algalisation trials were conducted through out India since late sixties and it was found that under varied agroclimatic conditions BGA biofertiliser can provide 25 to 30 Kg N per hectare per season in rice fields (Goyal and Venkataraman, 1971, Venkataraman, 1981). In addition to N, BGA biofertiliser enriches soil with extracellular carbohydrates, hormones, many other secondary metabolites and improves soil health. It increases the soil porosity, soil's water holding capacity, ameliorate degraded soil due to excessive use of chemical fertilisers and also the salt affected soils (Kaushik and Subhasini, 1985).

BGA biofertiliser technology for rice cultivation was developed in 1978 at IARI, New Delhi (Venkataraman, 1977) and is still taken up on a trial and error basis and not utilized to noticeable extent. Despite 25 years of research on BGA biofertiliser, our knowledge on their practical utilization seldom progressed due to several reasons: (1) Imbalance between laboratory and field studies, (2) Emphasis on grain yield as the only criteria in inoculation experiments, (3) Under estimation of potential of indigenous/region specific strains, (4) Little emphasis to find out the factors hindering the establishment of inoculated strains, (5) No strict regulation on quality assessment of biofertilisers supplied to farmers for inoculation, (6) Lack of proper knowledge on this technology among the extension staff and (7) Lack of coordination between the scientists engaged in refining the technology in universities and research institutions with government departments, extension officials and the farmers who use them in the field.

Therefore in the research programme at the Department of Biotechnology, Utkal

University, priority was given to field studies with emphasis on finding out the cyanobacteria occurring in the rice fields of different agroclimatic regions of Orissa, their composition, seasonal cycle and biomass production in relation to physicochemical factors of the rice field soils, development of region specific, ecologically adaptive and stress compatible strains as biofertiliser package for field inoculation, development of suitable carrier materials, monitoring the competitiveness with local strains for establishment, their N contribution and grain yield and also the nature of secondary metabolites that condition the soils. Since April 2000, with financial support of the Department of Biotechnology, Government of India on the "Biotechnology based programme for women and rural development" the focus was to transfer the technology from lab to land and to generate entrepreneurs in BGA biofertiliser production and marketing for income generation through self employment in the rural areas of Orissa state.

BGA Biofertiliser technology developed at Utkal University, Bhubaneswar

Through a coordinated research project supported by the Department of Biotechnology (1994-1998), Government of India, a survey was undertaken for the first time, of the rice fields of all the agroclimatic zones of Orissa state and isolated 120 species/strains of nitrogen fixing BGA(cyanobacteria) which are now being maintained in a germplasm collection since 1996. All these cyanobacteria have been documented with their strain history, e.g. places of collection, growth rate, nitrogen fixing capability etc. and grown in healthy condition since then with frequent transfers to fresh medium in the laboratory (Nayak *et al* 1996, Adhikary 1998). Based on their capability to tolerate several stress factors, e.g. salinity, pH, pesticides, commercial inorganic fertilisers, desiccation etc. (Rath and Adhikary 1995, Padhi *et al* 1997, Das and Adhikary 1996, Adhikary and Sahu 2000), eight cyanobacterial species belonging to *Anabaena, Nostoc, Calothrix* and *Aulosira*, of which some were free floating and others epiphytic forms, and also one species each under the same genus either from western part or coastal regions of Orissa were selected for field use. Protocols to grow them year round in bulk, in cemented tanks under polyhouses, using alternate cheap medium has been standardized. Performance of carrier based inoculum containing region specific strains have been tested in the field for 3 consecutive years which showed about 8 to 12 per cent increase in the grain yield together with improvement in the soil quality. The later was determined basing on the increase in soil porosity, water holding capacity of soil, build-up of organic carbon and microbial population of soil. The production/multiplication technology was refined and upgraded emphasizing on quality biofertiliser production with authentic region specific strains, year round production in polyhouses in the out doors without any contamination, and with use of several alternate carriers like rice straw, coir or soils, completely free from contaminating microorganisms.

For popularization of the technology and its transfer from lab to land, a pilot scale demonstration, production cum quality control facility for BGA biofertiliser was set up in the village Maniakati under Asurabandha grampanchayat of Surada block in the Ganjam district (Orissa) since April 2000 (Fig. 1&2). Quality BGA biofertiliser produced at the site (Fig.3) are distributed to farmers free of cost for wide scale field testing. The performance and fate of such inoculants was continuously monitored in inoculated fields. Several

training programmes have been conducted for farmers, agricultural officials at the grass root level and entrepreneurs including women at the project site in the village since last 4 years (Fig.4). Several articles have been written in regional language in science magazines and news papers of the state. Proceedings of such training programmes were published in local dailies for popularization. Activities at the project site in the village has also been telecasted in popular news channels, and also discussed in the state Assembly which helped in creation of awareness among the users.

Usefulness of BGA biofertiliser technology for providing self employment

- Initial results prove that it is a viable technology and can play important role in agriculture and rural development.
- Five Kg of soil based biofertiliser or 1 Kg of straw/coir based biofertiliser with requisite number of viable cyanobacteria is sufficient to use in one acre of land soon after transplantation of rice. Cost per Kg of soil based biofertiliser for selling will be Rs.15/- to Rs.20/- which can be easily affordable by small and marginal farmers.
- Farmers and entrepreneurs can produce quality cyanobacteria biofertiliser using the organisms and know how available at Utkal University or at the project site in the village, 200 Km away from Bhubaneshwar.
- Unemployed youth, women self help groups and NGOs can produce this biofertiliser for income generation. This technology can be adopted as a cottage industry in all the grampanchayats of the state for providing self employment.

Economics (Cost-benefit ratio of the technology):

1. Investment of about Rs. 50,000/- and working by self in an area of 0.1 acre (for erecting polyhouse) one can get monthly profit of Rs. 5,000/-.
2. Investing about 3 lakhs and employing one full time worker (including Rs. 3,000/- towards the salary per month) in an area of 0.2 acre , one can earn a net monthly profit of about Rs. 6,000/- to 7,000/- per month.
3. Investing about 25 lakhs in 1 acre area and employing 8 persons (one technical staff, one marketing staff, two production staff, two labourers and two watchman with salary @ Rs.5000/- to 10,000/- per month), one can get a net profit of about 20,000/- to 25,000/- per month.

Technical know how and the region specific nitrogen fixing cyanobacterial strains developed and maintained for production and use as biofertiliser for rice cultivation in the Orissa state is available at Utkal University.

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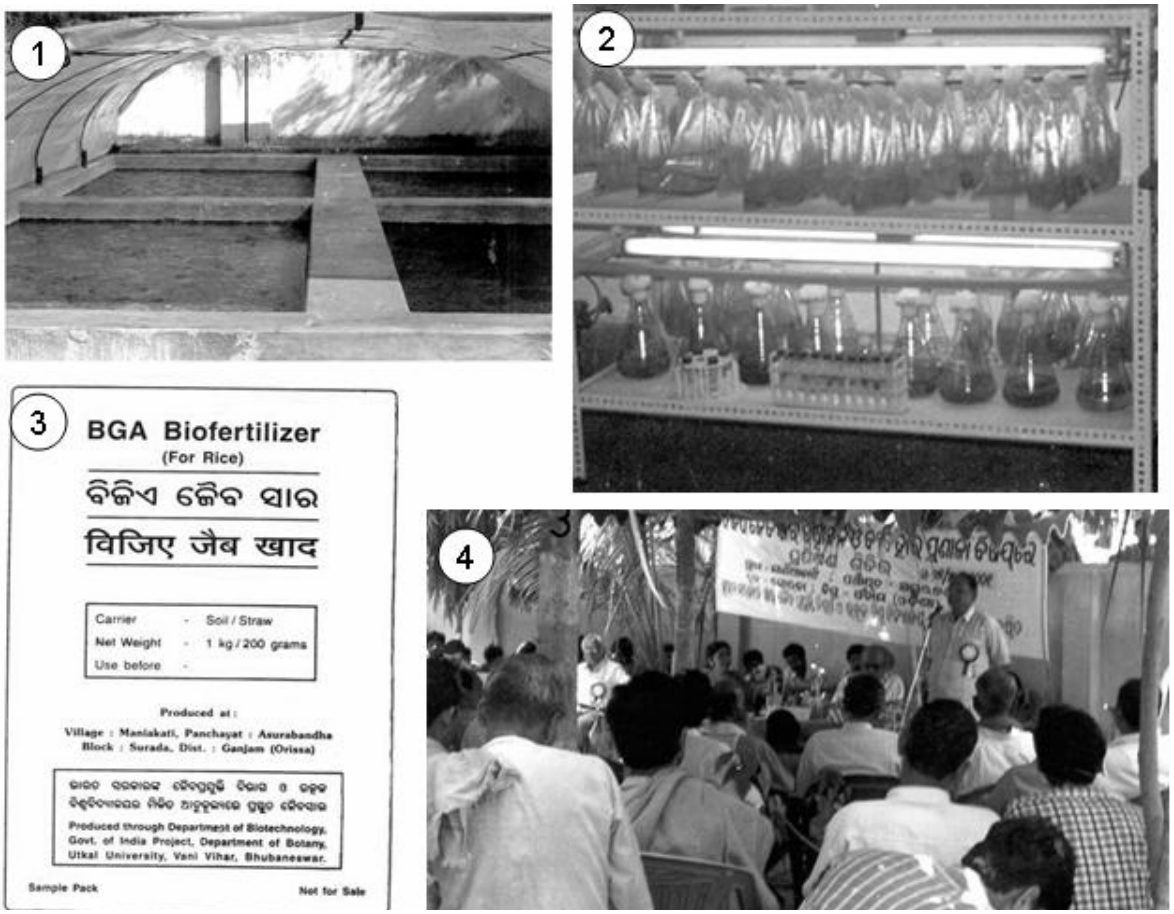

Fig. 1. BGA production tanks under polythene house. Fig. 2. BGA mother cultures under laboratory conditions. Fig. 3. BGA biofertiliser packet and
Fig. 4 Transfer of technology through farmer meet.

Effect of Inorganic Fertilisers and Biofertilisers on Productivity of Soybean

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सारांश

महाराष्ट्र राज्य के नागपुर जिले में किसानों के खेतों पर लिये गये एक तीन वर्षीय परीक्षण में सोयाबीन की फसल पर जैव उर्वरक व रासायनिक उर्वरकों के सम्मिलित प्रभाव की जाँच की गई। परीक्षण में पाया गया कि रासायनिक उर्वरकों की कुल अनुशंसित मात्रा (३० किलो नत्रजन, तथा ५६.२५ किलो स्फुर) के ७५ प्रतिशत के साथ ब्रैडीराइजोबियम जैपोनिकम तथा बैसिलस पॉलिमिक्सा के प्रयोग से सर्वाधिक ग्रथिं निर्माण तथा उत्पादन वृद्वि प्राप्त हुई। वर्षा का पूरे फसल समय में उपयुक्त वितरण कुल अधिक वर्षा के मुकाबले अधिक प्रभावी पाया गया।

Introduction

Although, soybean is an important cash crop in the central India, but its productivity is not up to the expectations, mainly because of the sub optimal population of native rhizobia under harsh pedo-edaphic environment. Soybean responds well to P fertilization and bacterial inoculation (Hajare *et al* 1994). To ensure optimum productivity and profitability of soybean production, it is essential to develop an integrated nutrient management system by involving bio and inorganic fertilisers. The technology, which is assessed by farmers through participatory mode, may have more acceptance by stakeholders. With this background present study was conducted at farmers' field to demonstrate the importance of integrated nutrient management practices (INMP) in typical black cotton soils of Nagpur (Kokarda village) under Institute Village Linkage Project (IVLP).

Materials and Methods

Experiments were conducted during Kharif 2000, 2001 and 2002 in farmers' field (Fine, smectitic, hyperthermic Vertic Haplustepts), at different sites of the field. The soil had pH of 8.3, $CaCO_3$ 8.4 per cent, organic carbon 0.7 per cent and Olsen-P 16.8 kg P_2O_5 ha^{-1}. Three treatments namely farmer's practice i.e. 2 bag DAP ha^{-1} (T1), recommended dose of fertilisers (NPK: 40:75:0) designated as T2 and 75 per cent of recommended dose of fertilisers (RDF) with *Rhizobium* inoculation as seed treatment and *Bacillus polymyxa* mixed with FYM (T3) as soil treatment were taken and replicated five times, in plot size of 1000 m^2. Necessary agro managements were carried as and when required.

Five plants were randomly uprooted from each plot after 30 and 60 days of sowing (DAS) and numbers of effective nodules were counted (expressed as mean of three years). Grain yield was recorded at harvest and computed for hectare.

Results and Discussions

The perusal of data (Table 1) indicate that yield increase due to T2 and T3 treatments ranged from 12.8 to 33.9 per cent and 21.1 to 44.9 per cent respectively over T1. As P requirement of the crop is not met in T1, application of 75 kg P_2O_5 ha^{-1} (T2) resulted in better root and nodule development (Table 1) and higher yield. The seed yield increased significantly in T3 compared to T1. Generally in this soil by

virtue of its intrinsic properties i.e. shrink-swell, calcareous and alkaline nature, the soluble P gets reverted to insoluble P (Finck and Venkateswarlu, 1982). Thus, application of *Bacillus polymyxa* resulted in solubilization of P that reflected in higher productivity of soybean irrespective of experimental years (Jagdish Prasad *et al* 1998) over other treatments. Further climatic environment i.e. super saturation of soil in rainy season followed by extreme desiccation in summer, work against the survival of native and introduced rhizobia. Under such circumstances, inoculation of seed with *Rhizobium* every year ensured the optimum population of effective rhizobia in rhizosphere leading to enhanced nodulation, nitrogen fixation and yield (Hajare *et al* 1994). The application of *B. polymyxa* as soil application and seed inoculation with *Bradyrhizobium japonicum* perhaps acted synergistically and enhanced the efficiency of 75 per cent of RDF over 100 per cent RDF (Ramamurthy *et al* 2001).

The variation in yield across the years is the reflection of distribution of rainfall rather than total rainfall as well as treatment (Table 2). The data indicate that during 2003 and 2000, there was very less rainfall in October which coincided with flowering and pod formation stage of crop and has severely affected the yield. Contrary to it, there was reasonably good yield in 2002 because of sufficient moisture in soil, due to rainfall in October.

Acknowledgement

Authors are thankful to the Regional Centre of Organic Farming (formerly Regional Biofertiliser Development Centre), Nagpur for providing the microbial inoculants.

Table 1. Nodulation and seed yield of soybean as influenced by integrated nutrient management practices.

Treatments	No. of nodules/plant (Mean of 3 years)		Seed yield (q/ha)			
	30DAS	60DAS	2000-01	2001-02	2002-03	Pooled
Farmers practice	20.1	34.0	7.1	10.9	10.9	9.6
RDF	26.0	55.0	9.2 (29.6)*	14.6 (33.9)	12.3 (12.8)	12.1 (26.0)
75%RDF+Rh+PSB	29.5	69.0	9.5 (33.8)	15.8 (44.9)	13.2 (21.1)	12.9 (34.4)
C.D.at 5 %	-	-	0.09	0.16	0.12	0.05

* Figures in parenthesis indicate percent increase over farmers practice

Table 2. Mean monthly rainfall during experimentation

Rainfall (mm)	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Total
2000	0.0	9.0	0.0	0.0	24.0	326.6	300.2	158.0	15.6	1.2	0.0	0.0	834.6
2001	0.0	0.0	36.8	42.6	3.2	222.8	162.0	282.0	43.0	105.0	8.6	0.0	906.0
2002	0.0	7.2	0.0	0.0	0.0	267.4	55.0	352.0	47.0	52.4	0.0	0.0	781.0

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The Centre for *Rhizobium* Studies (CRS)

CRS was launched in 1997 as a partnership between *The Grains Research and Development Corporation*, and three Western Australian Institutions *Murdoch University*, *Agriculture Western Australia* and *CLIMA* (Centre for Legumes in Mediterranean Agriculture) with the vision to provide a world focus for integrated research and education in the science of root-nodule bacteria and mission : "to strengthen the understanding and use of root-nodule bacteria in a Mediterranean environment". The key objectives of the centre are:

- Ensure all new and existing pasture and pulse legumes in southern Australia are provided with optimal inoculant root-nodule bacteria.
- Continue strategic research aimed at solving existing and potential threats to nitrogen fixation in the field.
- Continue basic research to increase our understanding of the ecology, physiology, biochemistry and molecular biology of root-nodule bacteria.
- Train students and visitors in the discipline of rhizobiology.

Evaluation of *Rhizobium* Inoculated *Acacia auriculiformis* in Degraded Acidic Soil

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सारांश

एक बंजर व अम्लीय भूमि में लिये गये प्रक्षेत्र परीक्षण में अकेसिया औरिकुलिफोरमिस पौधे पर राइजोबियम जैव उर्वरक के अकेले तथा रासायनिक उर्वरक के साथ प्रयोग से होने वाले प्रभाव की जाँच की गई। परीक्षण के प्रथम चरण में कर्नाटक व केरल राज्यों के विभिन्न स्थानों से चयनित राइजोबियम के २२ विभेदों की अम्ल सहन क्षमता तथा अकेसिया पौधे पर उनकी प्रभाविता की जाँच की गई। गमला परीक्षणों के आधार पर दो विभेद AR16 तथा AR19 का प्रक्षेत्र परीक्षण के लिये चयन किया। परीक्षण में AR19 विभेद का रासायनिक उर्वरक के साथ प्रयोग सर्वाधिक प्रभावी पाया गया। परीक्षण के परिणामों से अकेसिया पौधे के बंजर व अम्लीय भूमि में वृक्षारोपण के लिये उपयुक्त पैकेज तैयार करने में मदद मिलेगी।

Introduction

Nitrogen fixing trees are ideal species for aforestation of the degraded lands. Different species of *Acacia* have been found to be specifically useful in number of situations in tropics, not only in stabilization of land, but also in improving the fertility of the degraded soil (NFTA 1985, Prasad 1991). Although, N-fixing trees are known to establish and thrive in N-deficient degraded soils, the low pH conditions and non-availability of some vital nutrients such as P and Ca prove detrimental to their N-fixing potential. Low pH conditions not only restrict the proliferation of rhizobia but also affect the infection process, resulting into poor nodulation and poor N-fixation (Mengel and Schubert 1983, Sprent and Sprent 1990). The present study was aimed to evaluate the effect of specially isolated acidic pH tolerant strains of rhizobia on the growth and establishment of *Acacia auriculiformis* with and without chemical fertilisers in degraded acidic soils of Kerala.

Materials and Methods

A total of 22 rhizobial isolates were isolated from root nodules (Vincent 1970) of *Acacia auriculiformis* grown at 22 different sites in Kerala and Karnataka and were screened for their tolerance to acidity and aluminium stress (Ayanaba *et al* 1983). Acid tolerant strains were further tested for their symbiosis and N-fixation potential with *A. auriculiformis* seedlings grown in polybags under greenhouse conditions. Two best strains designated as AR-16 and AR-19 were selected for field trials on the basis of increase in dry weight, fresh weight of nodules and total N-content of plants using complete linkage cluster analysis (Everett 1974).

The selected experimental site was having a gentle slope. Soil was sandy loam in texture with very low pH (4.5), organic carbon (1.03%), nitrogen (0.085%), phosphorus (1.06 ppm extractable P), calcium (144 ppm extractable) and magnesium (20.8 ppm). Experiment was laid in RCB design with six treatments (R1, R2, R1F, R2F, F and control) and three replications. Each plot was planted with 49 seedlings in 7 x 7 pattern. Ten days old seedlings of *A. auriculiformis* raised in nursery beds were planted in polybags and

inoculated individually with 2ml of rhizobial suspension (22 x 10^6 cfu ml^{-1}). After 30 days of growth, these seedlings were planted in the experimental field. Fertiliser treatment plots were provided with urea and single superphosphate @ 10g each per plant after 30 days of planting.

Results and Discussion

Maximum increase in height and stem girth was found in *Rhizobium* inoculated plants provided with chemical fertilisers (Table 1). Although, both the *Rhizobium* strains showed increase in plant height, the strain AR-19 also increased the stem girth compared to control. The maximum height (174.3 cm) and stem girth (16.2 cm) was obtained in plots provided with chemical fertilisers and *Rhizobium* strain AR-19. Survival percentage among the planted seedlings was also found to be highest with *Rhizobium* strain AR-19+fertilisers The results indicate that although, *Rhizobium* alone are also beneficial to the plant growth, but the potential of this symbiosis can be harvested best with a little dose of chemical fertilisers. The improvement in symbiotic potential due to the application of fertilisers can be attributed to the better availability of phosphorus and availability of N at early nodulation stage. Doan (1994) has also demonstrated similar response on one year old out-planted trees of *A. auriculiformis* and *A. mangium*. Reinsvolt and Pope (1987) has also revealed that *Rhizobium* inoculated *Robinia pseudoacacia* along with the application of N and P increased all growth parameters and ARA of the nodules.

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Table 1. Effect of rhizobial inoculation on the growth of *A. auriculiformis* in degraded acid soil.

S.No.	Treatments	Plant height (cm)	Stem, girth (cm)	Survival percentage
1	*Rhizobium* AR-16	120.3^{D}	09.5^{C}	55.0^{A}
2	*Rhizobium* AR-19	118.6^{D}	09.6^{C}	58.3^{A}
3	AR-16+Fertiliser	161.0^{B}	13.8^{B}	65.3^{A}
4	AR-19+Fertiliser	174.3^{A}	16.2^{A}	77.0^{A}
5	Fertiliser alone	151.6^{B}	14.0^{B}	65.0^{A}
6	Control	135.9^{C}	09.9^{C}	51.0^{A}

Research Notes and New Reports

Evaluation of P-Solubilizing Activity by the Estimation of Left Over Insoluble P-Source – While studying P-solubilizing activity of soil microorganisms, the potential is generally gauged by estimating the quantity of available P in the medium and the quantity metabolized by the microorganisms. In all such cases the growth medium is provided with insoluble P-source. But in cases where P-solubilization potential is to be studied in the presence of available-P, the determination of solubilized phosphate in the medium is masked by the presence of relatively high concentration of soluble-P. To overcome the constraint Mikanova and Novakova (2002) suggested a modified method of determining the residual insoluble phosphate. In this method the test medium was subjected to filtration after incubation and the remaining insoluble phosphate (such as tri-calcium phosphate) was separated over filter paper. Medium was poured onto a filter paper and rinsed with hot distilled water (in order to remove slime and soluble phosphorus). Filter paper with remaining insoluble phosphate was dried for 15 min at 105^{o}C and then hydrolyzed with 2N H_2SO_4 for 8 hr. The solution was filtered again and made up to a specified volume. After necessary dilution, P was estimated spectrophotometrically.
(Source – Mikanova and Mikanova 2002 Rostlina Vyroba 48(9) : 397-400)

The Reporter Gene gfp Can be Used as a Marker for Monitoring the Strains of Biofertiliser Organisms – The cloning of gene encoding green fluorescent protein, gfp from jellyfish *Aequorea victoria* and its expression in *Escherichia coli* has attracted lot of attention from scientific community. The gfp gene is now being increasingly used as marker for monitoring the fate of inoculated test organisms in the natural environments. The advantage of gfp over other markers is that, unlike other bio-markers it does not require any substrate or additional cofactors for fluorescence.
(Source – Saha *et al* 2001 Current Science 80(5) : 669-671. Original reference – Chalf *et al* 1994 Science 263 : 802-805)

Interaction of Rhizobia with Cereals for Symbiotic Nitrogen Fixation – It is now generally agreed that the introduction of symbiotic nitrogen fixation into the cereals would be one of the most significant contributions biotechnology could make to the agriculture. Initial studies using maize interacting with *Parasponia* and *Aeschynomene* rhizobia indicated the likelihood, that the initiation of lateral root nodule formation in cereals and other non-legume crops, would establish a niche for symbiotic nitrogen fixation. The study of the interaction of *Azorhizobium caulinodans* (ORS571) with both rice and wheat has now shown significant levels of nitrogen fixation, as assessed by using acetylene reduction assays, and a correlation of this nitrogen fixation with the invasion of emerging lateral roots by 'crack entry' and the initiation of lateral root formation. ORS571 from lateral root nodules of *Sesbania rostrata* is known to have significant nitrogenases activity event at low levels of oxygen (2%). ORS310 which forms stem nodules on another tropical legume (*Aeschynomene indica*) is also known to have a nitrogenase that is tolerant to low levels of oxygen and has been found to be establishing maize roots and benefiting maize plants.
(Source – Cocking *et al* Cahiers Options Mediterraneennes, Vol 8 : 67-72)

Biofertilisers in Controlling Foot and Root Rot Diseases of Mungbean – In an micro-plot field experiment, carrier based *Rhizobium* inoculant on application to mungbean seeds as seed treatment was not only found to be increasing percentage seed germination and total dry weight of plants

but was also found to be reducing the incidence of foot and root rot diseases significantly. The experiment conducted with two mungbean varieties and two different commercial biofertilisers reduced the incidence of foot and root rot disease by about 69.6 and 76.7% in Binamoog-4 and by about 77.7 to 77.9% in Binamoog-3 varieties. (Source - Mohammad and Hossain 2003, Pakistan J. Plant Pathology. 2(2) : 91-96)

Effects of Different Salt Concentrations on the Growth of *Rhizobium* (Osmoadaptation) - Rhizobacteria, being soil microorganisms are confronted with fluctuating osmotic pressures of the rhizosphere. *Rhizobium* being an important rhizobacteria is affected by changes in salt concentration. In the present study, the author has studied the effect of varying salt concentrations from 0.2M to 0.00625M and as per the report, *Rhizobium* is capable of osmoadaptation and can tolerate high salt concentrations. The growth was found to be inversely proportional to salt concentration. In other words, growth decreases with increasing salt concentration. Rhizobial growth is more abundant at lower salt concentrations ranging from 0.00625M to 0.0125M. (Source –Rafiq S., www.mtsu.edu/~scientia)

Regulatory Mechanism for the Approval of New Inoculants in USA - Environmental Protection Agency (EPA), USA is the sole authority for granting necessary approval for the release of new substances including microorganisms. The new microorganisms are extensively reviewed by EPA pursuant to its authority under the Toxic Substances Control Act (TSCA). Section 5 of TSCA requires that information about the health and environmental effects of new chemical substances (including new microorganisms) be reviewed by EPA before the substances/ microorganisms are released for use commercially, in the United States. This review is conducted by EPA's Office of Pollution Prevention and Toxics. Information on new chemical substances or new microorganisms is submitted in the form of a pre-manufacture notice, or PMN. Each PMN for a new chemical substance or new microorganism receives a separate numerical designation and is evaluated on various risk factors. A subcommittee of the Biotechnology Science Advisory Committee (BSAC) meet to review the Agency's draft risk assessment. The BSAC is a panel of scientists from academia and other government agencies which functions as a peer review group for risk assessments of certain biotechnology products reviewed by EPA under TSCA. The important parameters considered for evaluation of rhizobial strains include: (a) Human, animal and plant health and exposure, (b) Antibiotic resistance (c) Effect on targeted host plant and other non-host plants, (d) nodulation and nitrogen fixation with host and non-host plants and (e) survival, dissemination and nodulation in environment. (Source-http://www.epa.gov/opptintr/biotech)

Sequencing of Symbiotic Gene of *Rhizobium* Strain NGR-234 - As a general rule, symbiotic genes are plasmid-borne in *Rhizobium* species and located on the chromosome in *Azo*(*Brady*)*rhizobium.* The strain *Rhizobium* sp. NGR234 is a highly interesting representative of rhizobia because it can live in symbiosis with more than 110 genera of legumes as well as the non-legume *Parasponia andersonii*, and possesses a large replicon, the sym-plasmid pNGR234*a,* which carries most symbiotic determinants including "nodulation" and "nitrogen fixation" genes. (Probably, this strain contains no second plasmid, but a ~5.7-Mbp chromosome!). The 536,165-bp sequence of pNGR234*a* has now been completely analyzed and released to the public domain. A total of 416 ORFs were predicted to encode proteins. In addition, 67 gene-fragments were identified that seem to be remnants of functional genes. Together with transcription studies, the sequence data point to novel symbiotic loci and signalling mechanisms.

(Source - Christoph *et al* 1996 Genome Res. **6** : 590-600).

Symbiotic *Rhizobium* and Nitric Oxide Induce Gene Expression of Nonsymbiotic Heamoglobin in *Lotus japonicus* - Authors characterized the expression profiles of *LjHb1* and *LjHb2*, nonsymbiotic heamoglobin (nonsym-Hb) genes of *Lotus japonicus*. Although *LjHb1* and *LjHb2* showed 77% homology in their cDNA sequences, *LjHb2* is located in a unique position in the phylogenetic tree of plant Hbs. The 5'-upstream regions of both genes contain the motif AAAGGG at a position similar to that in promoters of other nonsym-Hb genes. Expression profiles obtained by using quantitative RT-PCR showed that *LjHb1* and *LjHb2* were expressed in all tissues of mature plants, and expression was enhanced in mature root nodules. *LjHb1* was strongly induced under both hypoxic and cold conditions, and by the application of nitric oxide (NO) donor, whereas *LjHb2* was induced only by the application of sucrose. *LjHb1* was also induced transiently by the inoculation with the symbiotic *Rhizobium*, *Mesorhizobium loti* MAFF303099. A candidate gene for pathogen-inducible NO synthase (iNOS) of *L. japonicus* was also induced by inoculation with *M. loti* MAFF303099. Observations using fluorescence microscopy revealed the induction of *LjHb1* expression is corresponded to the generation of NO. These results suggest that nonsym-Hb and NO may have important roles in stress adaptation and in the early stage of legume-*Rhizobium* symbiosis.
(Source – Shimoda *et al* 2005, Plant and Cell Physiology)

Endophytic Colonization and In Planta Nitrogen Fixation by a *Herbaspirillum* sp. Isolated from Wild Rice Species - Nitrogen-fixing bacteria were isolated from the stems of wild and cultivated rice on a modified Rennie medium. Based on 16S ribosomal DNA (rDNA) sequences, the diazotrophic isolates were phylogenetically close to four genera: *Herbaspirillum*, *Ideonella*, *Enterobacter*, and *Azospirillum*. Phenotypic properties and signature sequences of 16S rDNA indicated that three isolates (B65, B501, and B512) belong to the *Herbaspirillum* genus. To examine whether *Herbaspirillum* sp. strain B501 isolated from wild rice, *Oryza officinalis*, endophytically colonizes rice plants, the *gfp* gene encoding green fluorescent protein (GFP) was introduced into the bacteria. Observations by fluorescence stereomicroscopy showed that the GFP-tagged bacteria colonized shoots and seeds of aseptically grown seedlings of the original wild rice after inoculation of the seeds. Conversely, for cultivated rice *Oryza sativa*, no GFP fluorescence was observed for shoots and only weak signals were observed for seeds. Observations by fluorescence and electron microscopy revealed that *Herbaspirillum* sp. strain B501 colonized mainly intercellular spaces in the leaves of wild rice. Colony counts of surface-sterilized rice seedlings inoculated with the GFP-tagged bacteria indicated significantly more bacterial populations inside the original wild rice than in cultivated rice varieties. Moreover, after bacterial inoculation, *in-planta* nitrogen fixation in young seedlings of wild rice, *O. officinalis*, was detected by the acetylene reduction and $^{15}N_2$ gas incorporation assays. Therefore, the authors conclude that *Herbaspirillum* sp. strain B501 is a diazotrophic endophyte compatible with wild rice, particularly *O. officinalis*.
(Source - Adel Elbeltagy *et al* 2001 Applied and Environmental Microbiology, 67 : 5285-5293)

Herbaspirillum lusitanum* sp. nov., a Novel Nitrogen-Fixing Bacterium Associated with Root Nodules of *Phaseolus vulgaris - Several bacterial strains were isolated from root nodules of *Phaseolus vulgaris* plants grown in a soil from Portugal. The strains were Gram-negative, aerobic, curved rod-shaped and motile. The isolates were catalase- and oxidase-positive. The TP-RAPD (two-primer randomly amplified polymorphic DNA) patterns of all strains

were identical, suggesting that they belong to the same species. The complete 16S rDNA sequence of a representative strain was obtained and phylogenetic analysis based on the neighbour-joining method indicated that this bacterium belongs to the β - *Proteobacteria* and that the closest related genus is *Herbaspirillum*. The DNA G+C content ranged from 57·9 to 61·9 mol%. Growth was observed with many different carbohydrates and organic acids including caprate, malate, citrate and phenylacetate. No growth was observed with maltose, *meso*-inositol, *meso*-erythritol or adipate as sole carbon source. According to the phenotypic and genotypic data obtained in this work, the bacterium represents a novel species of the genus *Herbaspirillum*, and the name *Herbaspirillum lusitanum* sp. nov. is proposed. The type strain is P6-12^T (=LMG 21710^T=CECT 5661^T).
(Source **-** Angel Valverde *et al* 2003 Int J Syst Evol Microbiol **53**, 1979-1983

Effective Microorganisms for Human Consumption – Recently a firm in US has claimed that for the first time ever they have developed an EM formulation named as Pro EM-1 for human consumption. So far this is the only EM-1 product made specifically for human consumption in the world.
(Source – US. EM News and Events.
www.emrousa.com/newsevents

Characterization of Nitrogen Fixing Bacterial Rhizosphere Communities using Restriction Fragment Length Polymorphism of PCR – Amplified *nif*H - Authors have developed a method to study the species diversity of N_2-fixing bacteria in the rhizosphere by examining the diversity of a segment of *nif*H, the conserved structural gene for dinitrogenase reductase. Total DNA was extracted from rhizosphere zones in natural and artificial sediments by bead-beating and purified by CsCl-EtBr gradient centrifugation. The average DNA yield was 5.5 µg g^{-1} of soil and was of sufficient purity for PCR amplification of *nif*H. Label ([-^{32}P] dCTP) was incorporated into the PCR reaction and *nif*H PCR products were restriction digested. Restriction Fragment Length Polymorphism (RFLP) analysis of the amplified sequences revealed differences in the community structure of N_2-fixing rhizobacteria of the salt marsh plant, *Spartina alterniflora*, and a laboratory cultured *Sesbania macrocarpa*. Soil inoculation experiments were used to determine the efficiency of the methods, and amplified *nif*H DNA could be detected when 10^4 cells each of *Vibrio natriegens* and *Azotobacter vinelandii* were added per gram of soil. These results indicate that RFLP analysis of amplified *nif*H sequences from rhizosphere communities may provide information on species composition and reveal shifts in diversity.
(Source -www.isb.vt.edu/brarg/brasym95/)

Seminar, Conferences, Symposia, Workshop, Fairs and Training Programmes

Proceedings of National Seminar-cum-Workshop on "Upgradation of Production Technology for Quality Production of Biological Inputs in Agriculture", held at Nagpur, during 3-4 March 2005 – A two days National Seminar-cum-Workshop on " Upgradation of Production Technology for Quality Production of Biological Inputs in Agriculture" was organized jointly by the Regional Centre of Organic Farming, Nagpur and Dr. P.D. Krishi Vidyapeeth, Akola at College of Agriculture, Nagpur during 3 – 4 March 2005. The major objectives of the seminar were: (a) To assess the current scenario in production and quality control (b) Identification of constraints and deficiencies (c) Alternative technologies available (d) Effective steps for quality production and (e) Finalization of draft standards for organic manures. The conference was attended by 155 scientists, Government officers, teachers, production unit representatives and NGO representatives from 12 states of the country.

The conference was inaugurated by Dr. K.S. Yavalkar, famous agricultural scientists of yester years and agro-input industrialist. Dr. Yavalkar in his inaugural speech emphasized that although, sufficient progress has been made in use of biological inputs, the time has now come to stress on the quality enforcement. Dr. P. Singh, Director, CICR and Dr. S.V. Sarode, Director Research, Dr. PDKV, Akola also emphasized the importance of quality and asked the industrial fraternity to come out with right quality product at right time and at right place. Three books were also released on the occasion. The technical deliberations were spread over five technical sessions and were mainly concentrating on (a) Biofertiliser production and quality control (b) New biofertilisers – production and quality control (c) Scientist-industry interface (d) Compost quality and framing of standards and (e) New BGA technology.

Abstract recommendations of the conference were as follows:

1. All production units should dispense with shake flask method using either glass flasks or bottles and introduce good quality fermenters.
2. Each unit must employ a qualified microbiologist
3. Each production unit must develop in-house quality monitoring facilities
4. Unsterile production should be replaced with sterile production system with proper automation
5. Each product must have total viable count $>1x10^9$ per gm or ml
6. All producers should obtain BIS certification. Conference unanimously recommended that Ministry of Agriculture, Govt. of India should take up necessary initiatives to either enforce a BIOFERTILISER CONTROL ACT or make it mandatory for all the production units to obtain BIS certification.
7. Suggested standards for organic manures and the one circulated by IISS, Bhopal be taken up as guidelines for judging the quality of compost
8. All state Governments should develop at least 2-3 quality control laboratories for organic manures, biopesticides, biofertilisers etc.
9. New inoculants such as *Gluconacetobacter* and PGPRs should be thoroughly tested in the fields before making wide claims.

10. In rice growing areas BGA inoculants should be promoted and BGA inoculant units be promoted at Taluka levels as per the technological details provided in the conference.

Proceedings of 13th Southern Regional Conference on Microbial Inoculants held at Agricultural College, Bijapur from 3-5, December 2004 - The XIII Southern Regional Conference on Microbial Inoculants (SRCMI) was held at Agricultural College, Bijapur from 3-5, December 2004. About 170 delegates from South India (Goa, Karnataka, Tamil Nadu, Kerala, Andhra Pradesh and Maharashtra) participated in deliberations spread over five technical sessions on the theme: "Microbes: Wheels of Organic Farming". Dr. S. Kannaiyan, Former Vice-Chancellor, TNAU, inaugurated the conference. In his inaugural remarks Dr. Kannaiyan expressed satisfaction on application of biofertilisers in farming system and as an important component in organic farming. Dr. S. A. Patil Hon'ble Vice-Chancellor, UAS, Dharwad presided over the inaugural function. In his presidential remarks, he opined that the present era is an era of Microbes and Microbiologists to boost up the crop production at large.

Dr.S. Kannaiyan and Dr. D.J. Bagyaraj were conferred with "**Life Time Achievement**" Award for their Yeoman's service to the field of Agricultural Microbiology and mankind in general. Dr. S.A. Patil, Hon'ble Vice-Chancellor, UAS, Dharwad presented the award with citation.

The following recommendations were made from the three days deliberations to pass on the information to Governing Council, Government and other agencies for their implementation.

1. Implementation of B.I.S. regulation act, quality standards for biofertilisers and commercial organic inputs.
2. Application of consortium of biofertilisers as microbial inputs for different crop plants.
3. Exchange of information on proven inoculants (super strains) to other Universities for research and extension purpose.
4. To formulate a detailed programme on quality parameters and yield components in different agro ecological regions.
5. To work out role of edaphic factors in responsive application of biofertilisers.
6. Extensive research work on formulation of quality control and use of liquid biofertilisers.
7. Biofertilisers and biocontrol agent's formulations should meet global standards in view of WTO implementation from April 2005.
8. Offering training programmes to the extension staff of SAUs and line departments for rapid transfer of technology on biofertilisers and biocontrol agents.
9. Urgent need to extend the work on PGPRs to different field crops like spice crops.
10. Application of methods of biopreservations particularly on onion and grapes exports.
11. Development of stress tolerant biocontrol agents to solve soil salinity problems.
12. Proper information on microbial biodiversity for identification of efficient biocontrol agents having disease suppression and biofertiliser production capacity.
13. It was proposed to form society of organic farmers and technocrats at Taluka and district levels.

(Source - Dr. A.B. Patil, Organizing Secretary 13th Southern Regional Conference

on Microbial Inoculants, Agricultural College, Bijapur, Karnataka)

Proceedings of "Interactive Workshop on Biofertilisers" held at Division of Microbiology, IARI, New Delhi during 5-6 November 2004 – A two days Interactive Workshop on Biofertilisers was organized at Division of Microbiology, Indian Agricultural Research Institute, New Delhi during 5-6 November 2004, under NATP project entitled "HRD in Biofertilisers" (TOE mode). The workshop was inaugurated by Dr. Mangala Rai, Director General of ICAR and Secretary, DARE, New Delhi.

Technical proceedings of the workshop were divided into four technical sessions pertaining to (a) Use of biofertilisers in agriculture (b) Marketing and distribution of biofertilisers: Constraints and Opportunities (c) Quality assurance in biofertilisers and (d) Recent developments in biofertilisers. Panel discussion was spread over two sessions devoted to (a) Current status, adoption and problems associated with biofertilisers and (b) Challenges before biofertiliser industry, role of Government and private sector.

Abstract recommendations of the workshop were as follows:

1. Government should take up necessary initiatives for the establishment of referral quality control laboratories in different states.
2. Appropriate technologies and strains be developed and made available to the users for effective benefits of technology to farmers in rain-fed and arid regions.
3. Higher shelf life products in both carrier based and liquid forms be promoted.
4. VAM technology should be promoted on priority and emphasis should be given to indigenously developed technology of TERI.
5. Promising bio-control traits of *Primiformaspora indica* should be tested under ICAR network.
6. Effective strategies are needed both at Government and at private level to increase the awareness about the potentiality of biofertilisers.
7. Government organizations should concentrate on developing specifications, quality control and price regulation.
8. Policy support is needed from Government side, so that farmers can get high quality product at affordable prices.
9. Inoculant industry needs to be regulated through registration and quality control regime.
10. Subsidy system need a relook and be targeted to improve product quality, so that manufacturers are able to produce high quality inoculants.
11. Commercial producers should be encouraged in private sector. There should be incentive for dealers.
12. Research institutions in Government sector should focus on research on new strain development, better formulations and improved inoculation methods.
13. The term biofertiliser is confusing and should be replaced with "Microbial Inoculants".
14. Viability of the new BGA technology has been demonstrated in some parts of the country. The model needs to be replicated in other relevant areas. The new technology holds promise for commercialization.

(Source – Dr Sunil Pabbi, Senior Scientist, NCCUBGA and Organizing Secretary, Interactive Workshop, Division of Microbiology, IARI, New Delhi)

Entrepreneurship Development Programme on Biofertilisers – A course on "Entrepreneurship Development Programme on Biofertilisers" was organized at IARI, New Delhi during 12-18 September 2004

under the project "HRD in Biofertilisers" and was sponsored by NATP. Dr. Sunil Pabbi, Sr. Scientist was course Director and Dr. Anil Saxena, Sr. Scientist was course coordinator. The participants included persons engaged in related activities, small scale biofertiliser producers and progressive educated farmers. The programme was divided into lectures, practical and demonstrations. The course content included – (a) Isolation, characterization and maintenance of inoculated strains (b) mass production and quality assurance of inoculants (c) Marketing and distribution of bio-inoculants.

10 days training courses on Production and quality control of Organic and biological Inputs – Under the National Project on Organic Farming, National Centre of Organic Farming, Ghaziabad (NCOF) and its three Regional Centre of Organic Farming (RCOF) at Bangalore, Bhubaneshwar and Nagpur organized each, a ten days "Training Course on Production and Quality Control of Organic and Biological Inputs". These courses were participated by large number of production and quality control technologists, NGO representatives and state Government officers associated with production and quality control of such organic inputs. Production and quality control aspects of various organic and biological inputs such as biofertilisers, biopesticides, biological control agents, organic manures, bio-gas slurry, vermicompost etc were taken up in detail through theory classes, practical demonstration, field visit and production facilities and laboratory visits. Details of each such programme conducted by various centres are as follows:

1. **By NCOF Ghaziabad** – The course was organized at NCOF, Ghaziabad during 15-24 February 2005. The training was attended by 20 participants representing different KVKs, KVIC and NGOs of Uttar Pradesh and Uttaranchal states.
2. **By RCOF Bangalore** – The course was organized in collaboration with and at Agro Clinic and Research Centre, Kottayam, Kerala, during 20-31st January 2005. The programme, was attended by 30 technical representatives of different organic input production units of Kerala state.
3. **By RCOF Bhubaneshwar** – The course was organized by RCOF Bhubaneshwar at Regional Fertiliser Quality Control Laboratory, Kalyani, W.B., during 11th to 20th January 2005. The training was attended by more than 20 participants from West Bengal state.
4. **By RCOF Nagpur** – The course was organized at RCOF, Nagpur during 1st March to 10th March 2005. The course was attended by 36 technical representatives of different production units, Krishi Vigyan Kendra and officers of Fertiliser Quality Control Laboratories of Maharashtra and Andhra Pradesh states.

Training course on organic farming at NCOF, Ghaziabad – Under the National Project on Organic Farming, National Centre of Organic Farming, Ghaziabad has also conducted two 10 days training course for Certification/Inspection agencies and service providers during 11-20th January and 02-11th February 2005 and one 8 days Model Training course on Organic Farming and Sustainable Agriculture during 15-22 March 2005 at NCOF, Ghaziabad. All these programmes were attended by representatives of different certification agencies, service providers and officers of different state Governments from all over the country. In all these programmes biofertilisers were discussed and projected as important component of nutrient management in organic farming.

Book Review

A Handbook of Biofertilisers (2004) By L.L. Somani, Ambica Book Agency, New Delhi, price Rs. 2050/-. – The book entitled "A Handbook of Biofertilisers" deals with the prospects and potential of different biofertiliser organisms such as *Rhizobium*, *Azotobacter*, *Azospirillum*, *Beijerinckia*, cyanobacteria, *Clostridium*, *Frankia* and *Azolla*. The compilation elaborates various details pertaining to their important aspects like occurrence, distribution, life cycle, characterization, morphology, nitrogen fixation, host specificity and factors affecting their growth and nutrient mobilization potential. Besides nitrogen fixers, the book also deals with the details of phosphate solubilizing microorganisms and elaborates their important aspects like mineralization of organic and inorganic phosphorus, mechanism of phosphate solubilization and their potential as plant growth promoter. Vesicular and arbuscular mycorrhizal fungus (VAM) has received adequate attention and gives in-depth information on their role and potential in improving nutrient and water uptake by host plants. In brief the book is an excellent compilation of all the important organisms, which have proven potential in nutrient mobilization, incorporating the up to date status of knowledge and technology. (AKY)

Biological Inputs in Agriculture (2005), Edited by A.K. Yadav, Sarita Mowade, V.Y. Deoghare and A.K. Shukla, Published by Regional Centre of Organic Farming, Nagpur. Released on the occasion of the National Seminar-cum-Workshop on "Upgradation of Production Technology for Quality Production of Biological Inputs in Agriculture". p-122. (Free publication-Copies can be obtained from RCOF, Nagpur) – The book is basically a compilation of papers contributed and presented by eminent scientist of the country in the field of biofertilisers and other biological inputs such as composts and organic manures in National Seminar-cum-Workshop held at Nagpur during 3-4 March 2005. The book is divided in 12 chapters. First three chapters deal mainly with the current status and problems facing the biofertiliser industry today, quality control scenario of biofertilisers in general, in the country and Maharashtra and Andhra Pradesh in particular. Among the new biofertilisers, PGPRs for different crops and *Gluconacetobacter diazotrophicus* for sugarcane have been found to be very promising. Detailed production technology with different steps of organism's culture, identification, multiplication and inoculant preparation has been summarized in fourth and fifth chapter. Sixth chapter deals with the gaps in earlier BGA inoculant production technology and summarizes a new cyanobacterial inoculant production technology, under which organisms are cultivated under controlled conditions and a low volume product of consistently good quality can be obtained. The technology holds good promise for mass scale commercialization. An appraisal of biofertiliser's response in field trials conducted over 10 years, evaluation of composts from quality point of view and technologies available for fast identification and contamination detection in production process have been discussed in the subsequent chapters. The editors hope that the compilation will not only be useful for the technical personals of biological input producers, but will also be an purposeful reckoner for all those who study, teach, research and plan for the future and sustainability of such environment friendly commodities. (PB)

Publications available with NCOF and RCOFs on sale/demand

1. Biofertiliser Newsletter – Biannual (Free circulation). (Back volumes since 1996 available with NCOF).
2. A Book on Biofertiliser for Extension Workers (1995) by P. Bhattacharyya & U.C. Mishra Cost Rs. 125/- (available with NCOF and all RCOF's)
3. जैव उर्वरक विस्तार प्रशिक्षण पुस्तिका (1997) द्वारा पी. भट्टाचार्या एवं यू.सी मिश्रा मूल्य रु. 125/- (available with NCOF and all RCOF's)
4. Three decades of Research in Biofertilisers and Organic Farming in North East India. (2001) by A.K. Yadav and S. Raychaudhuri. (available with RCOF, Nagpur, Also available in CD)
5. Souvenir I and II NER Conference on Biofertilisers (1999 and 2001) by A.K. Yadav and S. Raychaudhuri (available in CD only)
6. Introducing Biofertilisers in the North Eastern Region – A manual for Extension Workers. (1999) by A.K. Yadav and S. Raychaudhuri. (available with RCOF, Nagpur, Also available in CD)
7. Use and Development of Microbial inoculants (1999) by T. Singh, A.K. Yadav and S. Raychaudhuri. (available with RCOF, Imphal)
8. Organic Farming –A Ray of Hope for Indian Farmers. (2004) By A. K. Yadav and Sarita Mowade. Edited by P. Bhattacharyya, Pub. V.B. Foundation Pune. (Available with R.C.O.F. Nagpur)
9. सेंद्रिय शेती - उज्ज्वल भविष्याचा किरण (२००४) ए. के. यादव व सरिता मोवाडे, संपादन डा. पी भट्टाचार्या, क्षेत्रीय जैव उर्वरक विकास केन्द्र, नागपुर (available with RCOF, Nagpur)
10. Proceedings of National Conference on Quality Control of Biofertilisers (2004) Edited by P. Bhattacharyya and V. Dwivedi. Publ. NBDC, Ghaziabad. (available with NCOF, Ghaziabad)
11. Organic Food Production in India- Status, Strategy and Scope (2004) By P. Bhattacharyya, Agrobios (India). Cost Rs. 495/- p-182 (Available with publisher)
12. Catalogue on Biofertiliser's strains having detailed information on various promising biofertiliser strains being used in India (available with NCOF)
13. Biofertiliser Scenario of Andhra Pradesh – A Documentary Report. by P. Bhattacharyya. (available with RCOF, Nagpur)
14. Biofertilisers in Maharashtra and Goa – Documentary Report. by P. Bhattacharyya (available with RCOF, Nagpur)
15. 1050 Crop Demonstrations on Biofertilisers by M.R. Motsara and R.N. Bisoyi. (available with NCOF)
16. Recent Advances in Biofertiliser Technology (2001) by A.K. Yadav, M.R. Motsara and S. Raychaudhuri. Published by SPURT, New Delhi Cost Rs. 1100/- (available with publisher).
17. A Refresher Course Manual on Biofertilisers (2000) by T. Singh, T.K. Ghosh, M.K. Tyagi and J.S. Duhan, RBDC, Hisar (RCOF, Hisar)
18. Biofertiliser Situation in Orissa (1999) by K. Chandra *et al* RBDC, Bhubaneshwar (available with RCOF, Bangalore in CD)
19. Biofertiliser Vision 2000 (2000) by K. Chandra *et al* RBDC, Bhubaneshwar (available with RCOF, Bangalore in CD)
20. Biotechnology in Sustainable and Organic Farming-Scope and Potential, (2004) Edited by A.K.Yadav, S. Raychaudhuri and N.C. Talukdar. Shree Publisher and Distributors, New Delhi. Cost Rs.1500/-(Available with publisher)
21. Potentialities of Organic Farming in India (2003) by R.N. Bisoyi, RBDC, Bangalore (Available with R.C.O.F. Bangalore).
22. Biological Inputs in Agriculture (2005) Ed. A.K. Yadav, Sarita Mowade, V.Y. Deoghare and A.K. Shukla. RCOF, Nagpur (Free publication available with RCOF, Nagpur).
23. सेंद्रिय शेती - समृद्धि कडे वाटचाळ (२००५) ए. के. यादव व सरिता मोवाडे, क्षेत्रीय जैविक खेती केन्द्र, नागपुर (available with RCOF, Nagpur)