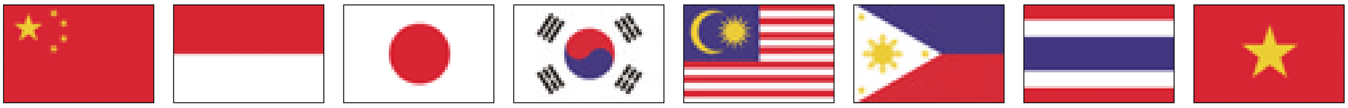
*FNCA (Forum for Nuclear Cooperation in Asia) Biofertilizer Project*



# FNCA Biofertilizer Newsletter

**Issue No. 7**
**February 2007**

## Message from the Philippines

*Dear Readers,*

This 7th issue of the FNCA (Forum for Nuclear Cooperation in Asia) Biofertilizer Newsletter features the activities pertaining to research and development, industry, use and promotion and impact of Biofertilizers in Philippine agriculture. It's my pleasure to share to you information herein that you may find interesting and valuable in line with the same activities in your respective areas.

The need to address the food requirement of the growing population in the Philippines made the farmers adopt the latest technology in agriculture like the use of high yielding and resistant crop varieties. These varieties however require high amount of nutrients making the farmers dependent on agricultural input like inorganic fertilizers. It was reported that from 1966 to 1998 fertilizer application in the Philippines increased from 20% to 80%. Nitrogen, considered as the most limiting factor in crop production, gains the highest mark of all the major nutrients from inorganic sources applied by farmers. The 2001 FAO (Food and Agriculture Organization) Yearbook on fertilizers stated that from 1987 to 1998, Philippine consumption of nitrogenous fertilizers increased from 371,487 MT to 546,499 MT. Such consumption resulted to high production for some time but adverse soil and other environmental conditions were observed in the long run. This called for the use of other alternative inputs. The use of biofertilizers has been determined as one of the main options to address the rising concern on agricultural and environmental sustainability.

Biofertilizer industry in the Philippines grown considerably with the promotion of Government of bioorganic farming and the active involvement of research institutions, private individuals and enterprises. Biofertilizers in various forms have been developed and more researches on rhizobia legume symbiosis, azolla, mychorriza, sesbania, blue green algae and the living microorganism (N-fixing bacteria) were encouraged.

My sincere thanks to the contributors who graciously contributed some highlights of their research works and efforts. Without their contributions, this issue would not have materialized.

Best regards,

*Richard M. Balog*
FNCA Biofertilizer Project Leader, Philippines

# FNCA (Forum for Nuclear Cooperation in Asia) 2005 Biofertilizer Workshop

The 2005 FNCA Biofertilizer Workshop was held last 9-13 January 2006 at the PNRI (Philippine Nuclear Research Institute), Quezon City, Manila jointly hosted by the DOST (Department of Science and Technology) - Philippines and MEXT (Ministry of Education, Culture, Sport, Science and Technology) - Japan. It was attended by 17 participants, primarily country project leaders from Indonesia, Japan, Korea, Malaysia, Philippines, Thailand and Viet Nam. Local participants included PNRI collaborators from Biotech (National Institute of Molecular Biology and Biotechnology) - UPLB (University of Philippines Los Banos) and DA (Department of Agriculture) - BSWM (Bureau of Soils and Water Management). The workshop was highlighted by the presence and messages of key persons of the host organizations in person of DOST Secretary Dr. Estrella Alabastro, PNRI Director Dr. Alumanda Dela Rosa and Director for International Nuclear Cooperation of MEXT Ms. Takiko Sano. The workshop activities comprise of presentation of country reports by the respective project leaders, roundtable discussions of project accomplishments/activities and plans, special lectures by invited lecturers and technical visits to biofertilizer facilities.

*The workshop participants with Dr. Alumanda Dela Rosa (sitting 2nd from left), Dr. Estrella Alabastro (sitting 3rd from left) and Ms. Takiko Sano (sitting 4th from left).*

*Visit to the Bio-N production facility at Biotech-UPLB. Supervising the facility were Dr. Mercedes U. Garcia (standing 4th from right) and her staff Ms. Juliet Anarna (standing center in front). Inset: inside the facility.*

Dr. Mercedes U. Garcia of the National Institute of Biotechnology-UPLB lectured on the "Role of Bio-N in Ginintuang Masaganang Ani Program for corn in the Philippines". Dr. Gina P. Nilo of DA-BSWM lectured on Organic-Based Agriculture "Agri-Kalikasan" Program/Tipid-Abono Program in the Philippines.

The participants visited Biotech-UPLB and Bio-N Mixing Plant at Barangay Lawy, Capas, Tarlac. The participants were given overview about the Biotech particularly pertaining to its developed biofertilizers under the program on agriculture and forestry. The participants also visited the Bio-N production facility of the institute where they were informed about the processes of the Bio-N inoculant production. The facility, supervised by the inventor of Bio-N Dr. Mercedes U. Garcia and her staff Juliet A. Anarna, was established through the funds provided by the DA in 2003 for the development and application of Bio-N on high value crops.

The establishment of mixing plants is one of the strategies in the promotion of biofertilizer use by farmers which was a big help for sus-

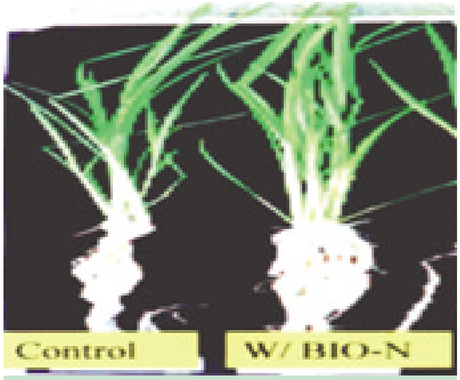
*Mr. Tolentino, chairman of the St. Philip PMPC talked to the participants about the cooperative and the mixing plant. (Inset: the participants in discussion about how the mixing plant operates).*

tainable crop production in the locality. The mixing plant visited was operated by a farmer's cooperative St. Philip PMPC (Primary Multi-Purpose Cooperative) Inc. in collaboration with the Biotech-UPLB. The Bio-N mixing plant was also established in cooperation with the DA-RFU (Regional Field Unit) 3-Regional Soils Laboratory, Tarlac LGU and PDA-Tarlac.

## Biofertilizer studies conducted by PNRI under FNCA framework

The biofertilizer used in the study conducted by the PNRI under the FNCA framework was Bio-N, developed by Dr. Mercedes U. Garcia of the Biotech-UPLB. Though various forms of biofertilizer have been developed in the country, Bio-N got the nod from the government as the most promising technology in crop production particularly in reducing the use of chemical fertilizers. The government, in line with its program on sustainable agriculture allocated funds for the mass production of Bio-N as this was foreseen to greatly benefit the farmers.

Bio-N is an inoculant in solid powder form that contains two species of the nitrogen fixing bacteria Azospirillum isolated from he roots of a local grass talahib (Saccharum spontaneum L.) which have been screened for their effectiveness to variety of agricultural crops. It can fix atmospheric nitrogen and transform it into form usable by plants or crops. Beneficial effects of Bio-N includes enhancement of shoot growth and root development, makes plant resistant to drought and pest attack, reduces incidence of rice tungro and corn earworm infestation and increases yield and milling recovery of rice and corn. It was observed to provide approximately 30%-50% of the nitrogen required by crops.




*Root development of rice as affected by Bio-N inoculation*

*Bio-N Biofertilizer*

## Method of Bio-N application on crops

*Bio-N application on corn as seed coating*

*Bio-N application on rice as root dip*

Bio-N is generally applied as seed coating for corn or as suspension for use in watering seed beds or root dips for rice. The use of 5-6 packets (200g per packet) of Bio-N per hectare is recommended to inoculate rice or corn.

For corn, seeds are moistened with water and mixed with sufficient amount of inoculants until seeds are evenly coated. A sticker could be use to enhance adsorption of Bio-N to the seeds. Coated seeds are sown immediately and exposure of the inoculated seeds from direct sunlight should be avoided.

For direct-seeded rice, germinated seeds (with embryonic roots sprouted) are coated with the inoculant and sown directly to the field or on prepared beds. For transplanted rice, Bio-N is mixed with water to form slurry. Roots of seedlings are pruned into uniform length and dipped into the slurry for at least 30 minutes to an hour before transplanting.

## Field experiment and yield trials

Since the development of Bio-N in 1985, field demonstration trials have been conducted to test its efficacy to various crops.

The Biotech, as the primary institute for the development of Bio-N conducted and supervised several field trials and supervised farmer managed field trials for assessment on effect of Bio-N to crops. In the study conducted on the effect of Bio-N and inorganic fertilizer on the yield of corn (Super sweet var.) in aBio-N application has higher yield than application of inorganic fertilizer and was comparable to the yield when application of inorganic fertilizer was cut into half plus Bio-N. This further showed that the use of Bio-N has the least value of input (in peso) per yield (kg) which indicates that farmers can save big amount of fertilizer expenses through the use of Bio-N.

The study conducted by the PNRI on the use of nuclear techniques in the assessment of Bio-N fertilizer as seed inoculant for corn on low fertility area in the province of Isabela (around 400km north of Manila) showed that corn yield was only 208 kg/ha (kilogram per hectare) when not inoculated with Bio-N and without fertilizer application. Corn yield was increased tremendously to 2,880kg/ha when

Table 1. Effect of Bio-N and inorganic fertilizer on the yield of corn (Super sweet var.) in farmers field demo, dry season 2001.

| Treatment | Rate of application (kg/ha) | Yield (tons/ha) | Cost of fertilizer input (P) | Input (P) per kg yield |
|---|---|---|---|---|
| Chemical (N-P-K) | 90-60-60 | 8.87 | 4,371 | 0.433 |
| 50% chemical + Bio-N | 45-30-30 + 5 packets | 10.49 | 2,336 | 0.233 |
| Bio-N | 5 packets/ha | 9.40 | 150 | 0.016 |

(Source: Garcia, 2005)

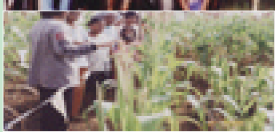
*Field experiment on corn (with yield levels) in low fertility area at Isabela province.*

inoculated with Bio-N plus application of P and K fertilizer. This resulted to an increase in income from PhP1,456.00 to PhP14,341.50. Bio-N inoculation plus application of half and full recommended rate of N fertilizer yielded 3,460kg/ha and 2,960kg/ha, resulting in increase in income of PhP16,230 and PhP10,558 respectively. The experiment also used N-15 to quantify biological nitrogen fixing capacity of Bio-N. N-15 data indicated that 19-24% of the nitrogen taken by the plants is nitrogen from the atmosphere that is fixed by the Bio-N. (Please also see related article by Biotech on biofertilizers)

## Promotion of biofertilizer use by farmers

Transfers of technology on use of Biofertilizers in line with various initiatives and efforts in promoting organic agriculture still remains an enormous challenge in Philippine agriculture. Despite of the numerous discoveries and technologies on biofertilizer, bringing it to the end users particularly the farmers is a difficult task. Convincing farmers is costly as it entails unlearning and getting rid of their old habits of using chemical fertilizers. Different means of has been undertaken in the Philippines in its continuous endeavor of promoting the use of biofertilizers. Such means includes conduct of exhibitions and trade fair, consultations, symposia, trainings, field demonstrations, and tapping broadcast and print media for information dissemination.

One of the recent and remarkable efforts by the government in promoting the use of the biofertilizer Bio-N was the establishments of

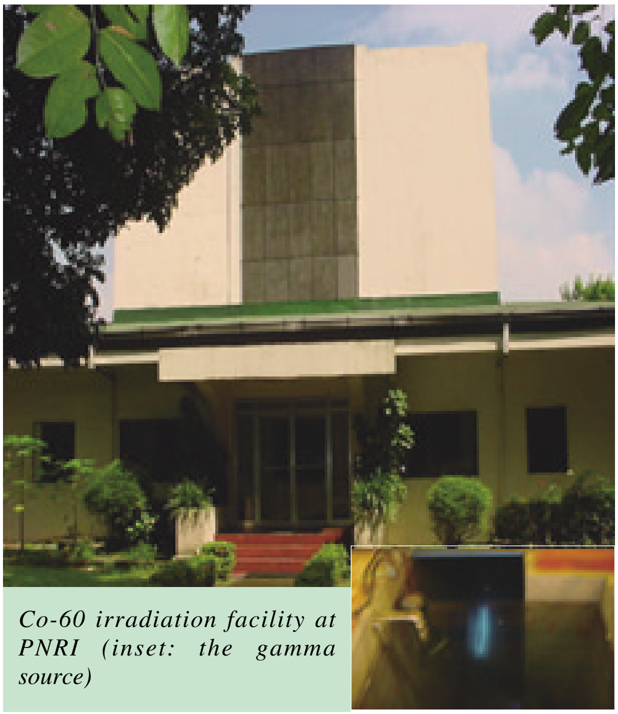



*A pose with the farmers during "Farmers Field Day" in Isabela province. (Inset: explaining to farmers about the experiment.)*


*One of the Bo-N mixing plants established in province of Cotabato. (inset:: Giving technical assistance to the recipient)* mixing plants. The establishments of mixing plants is one of the projects being undertaken by the DA Ginintuang Masaganang Ani Corn Program, in collaboration with the Biotech-UPLB, LGU's (Local Government Units) and farmers organization/cooperatives in respond to the farmers increasing demand of the Bio-N in late 1996. The mixing plants are considered satellite production and sales centers and at the same time served as distribution channels that made Bio-N accessible to the local consumers especially the farmers who were sourcing it from UPLB. There have been 32 mixing plants strategically established throughout the country that produces and sold Bio-N at price affordable to farmers. (Please also see related article by Biotech on promotion and technology transfer of Biotech biofertilizers.)

## Application of gamma irradiation for sterilization of biofertilizer carriers

Biofertilizer primarily contains microbial inoculants usually proliferated in a nutrient source medium called as carriers. In the production of biofertilizers, carriers undergo a process of sterilization to create a favorable environment for the growth of desired inoculants free from antagonistic microbes. Conventional method of sterilization of carriers for biofertilizers is done through the use heat-autoclave. This sterilization process poses some disadvantages such as incomplete sterilization, may result to some changes in physical and chemical properties of carriers, costly and suitable only to small scale treatments. Alternative means of sterilization process has been found in the utilization of the killing effect of gamma rays to microorganism. This process has been undergone in the Philippines in line with its activities in improvement of the quality of biofertilizers. The PNRI operates a multi purpose Co-60 gamma irradiation facility, which is the only one in the country. The application of gamma irradiation for sterilization of biofertilizer carrier however is still in its experimental stage. Bio-N for instance, whose carrier is compose of soil and charcoal, gamma irradiation showed decrease of microbial load at increasing dose and is sterilized at the dose 30kGy. Different biofertilizers however have different carriers so further experimentation is needed and sufficient data should be acquired especially on the advantages of the gamma irradiation compared to conventional methods to convince the biofertilizer producers to adopt the application of gamma irradiation for sterilization of biofertilizer carriers.



*Co-60 irradiation facility at PNRI (inset: the gamma source)*

## Researches on BIOFERTILIZERS in the Bureau of Soils & Water Management, Deapartment of Agriculture, Dilliman, Quezon City, Phillippines

# ENHANCEMENT OF BIOLOGICAL NITROGEN FIXATION IN LEGUMES TO INCREASE PRODUCTIVITY OF ACID UPLAND SOILS

**M. J. Palis, A. O. Yambot and J. S. Rojales**

### Rationale:

Marginal soils occupy a large area in the Philippines. The acid upland soil is estimated to be 17 million hectares or roughly equivalent to 58 % of the total land area of the country (Atienza, 1991). These soils are generally characterized as highly weathered soils with low productivity (IBSRAM, 1987).

Despite the widespread belief that acid upland soils cannot support intensive and sustained agriculture, there are ample evidence that they can be continuously cultivated and intensely managed for growing annual and perennial crops. To sustain crop production in these soils, inclusion of legumes in the production systems had been advocated. These legumes can serve as nitrogen sources and their use by farmers can reduce the need for nitrogen fertilizers. However, growth and nitrogen fixation of legumes in acid soils are constrained by a number of soil factors which include toxicities of Al, Mn and other metal ions (Zn, Cu, Cd), low soil pH, deficiencies of Ca, Mg, P, Mo and other elements including N and K. To successfully grow legumes in acid soils requires soil management strategies such as choosing legume species that show tolerance to soil acidity, liming and addition of fertilizers and organic matter.

This study aims to determine the effects of lime, phosphorus and micronutrient applications on the growth, nitrogen fixation and yield of legumes and to determine the increase in productivity with the application of these inputs.

### Methodology:

The experiment was established in Kapatalan, Siniloan, Laguna, around 106 kilometers south of Manila. The soil in the experimental area was taxonomically classified as Fine Isohypothermic, Typic, Kanhapludult ( Evangelista, pers.com) with a pH of 4.4 and 12.6 ppm available P.  Peanut (cv. UPL-Pn2) and mungbean (cv. MG-50 L Yellow) were used as test crops.

The study had the following treatments:

a. Lime application
   - with lime (5 tons $CaCO_3$/ha)
   - without lime
b. Micronutrient application
   - with micronutrient (50 kg fused trace elements/ha)
   - without micronutrient
c. Phosphorus application (kg/ha)
   - 0,40,80,120,160

A split-plot design was used with liming as the main plot, micronutrient application as the sub-plot and phosphorus levels as the sub-sub-plot. The treatments were replicated three times. Nitrogen and potassium fertilizers were applied at the rate of 20 kg N/ha and 30 kg K20 /ha. Rhizobium inoculants for peanut and mungbean were inoculated onto the seed prior to planting.

Figure 1. General view of the experiment.

Figure 2. Researchers preparing samples for acetylene-reduction assay.

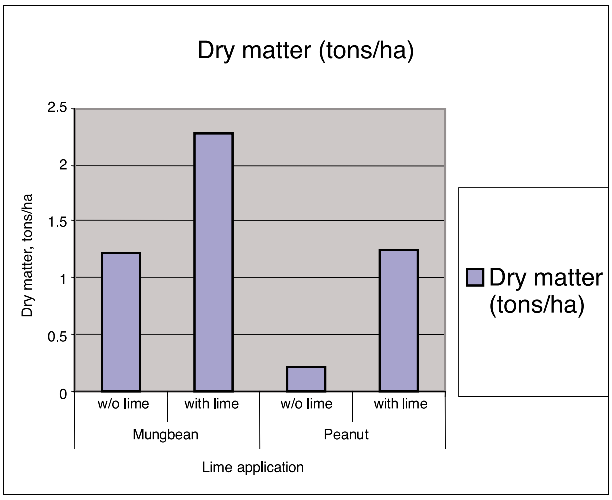
The plants were grown to maturity. At near blooming stage (7 weeks after planting), 10 representative plants from the sampling area were obtained for determination of dry matter yield, acetylene-reduction activity and nodule weight. The general view of the experiment at maximum vegetative stage is shown in Figure 1. Preparation of samples for acetylene-reduction assay is shown in Figure 2.

Harvesting of the plants was done at physiological maturity. All pods from the plants in the harvest area were obtained. The pods were sun-dried, shelled and the weight of seeds was taken.

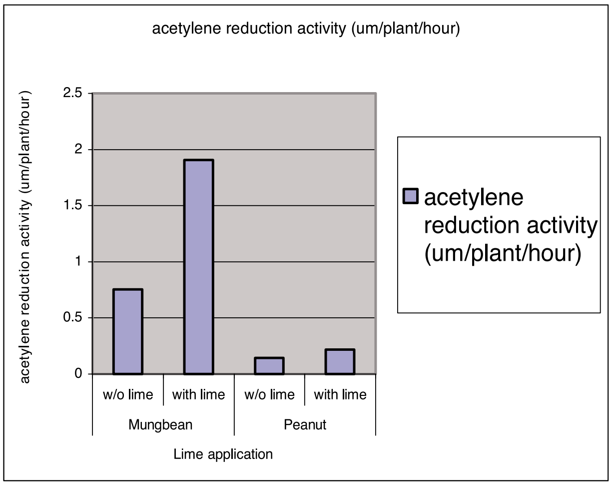
A simple cost and return analysis was also done after harvest to determine whether growing legumes in acid soils is profitable for the marginal farmers.

## Salient Findings:

1. The application of lime at the rate of 5 tons CaCO3 /ha gave a marked increase on the dry matter, nitrogenase activity, nodule weight, and pod and seed weight of both peanut and mungbean as shown in Figures 3-6.

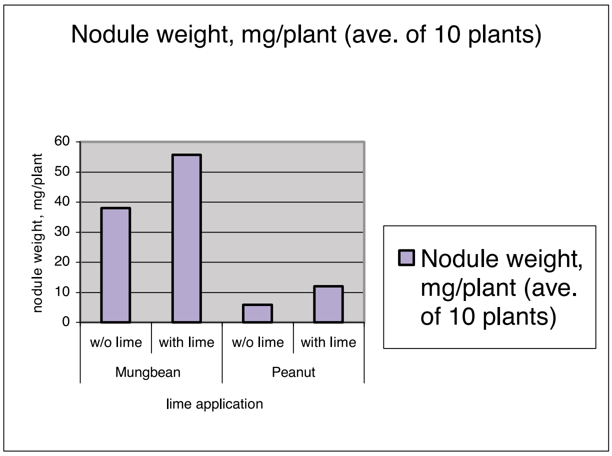


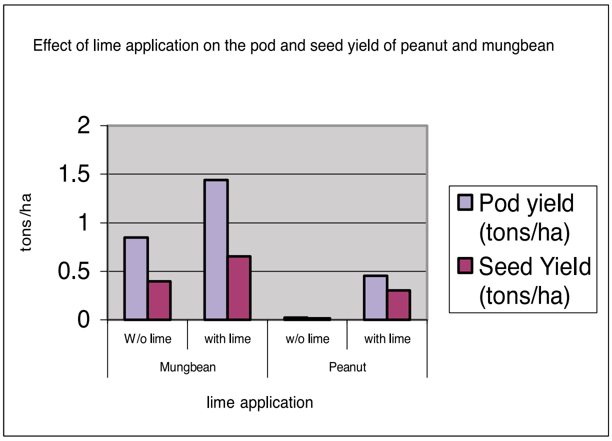






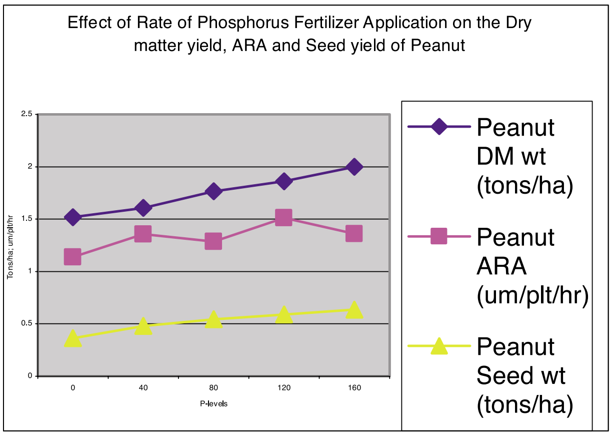
Figures 3 and 4. Effect of liming on the dry matter and acetylene-reduction activity of peanut and mungbean grown in an Ultisol.

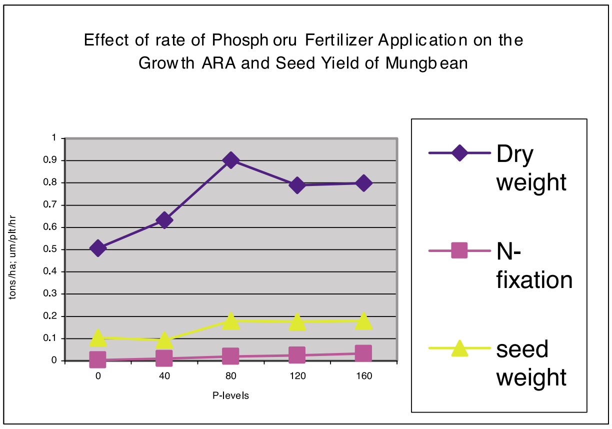



Figures 5 and 6. Effect of liming on the nodule weight and pod and seed yield of peanut and mungbean grown in an Ultisol.

2. Phosphorus application likewise increased the growth, nitrogen fixation and yield of peanut, but the increase was not as pronounced as when compared to the effect of lime (Figures 7 and 8).




Figures 7 and 8. Effect of rate of phosphorus fertilization on the growth, nitrogen fixation and yield of peanut and mungbeans grown in an Inceptisol.

3. Based on simple cost and return analysis, growing of mungbean in acid soils could not be recommended to farmers. A net income of P21,563.00/ha (USD 431.26) can be obtained with planting of peanut in acid soils with the application of 5 tons lime, micronutrient, and 40 kg of P2O5 per hectare.
4. Peanut seemed to be more tolerant to soil acidity when compared to mungbean.

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# SELECTION OF COMPETITIVE STRAINS OF RHIZOBIA FOR MUNGBEANS IN MARGINAL SOILS

**M. J. Palis, A. O. Yambot, J. S. Rojales, V. F. Naboa, S. Arai, H. Ito and M. Nakamura**

## Introduction:

A commonly used technique to improve legume growth and seed yield is inoculation with useful Rhizobium strain with a high potential for nitrogen-fixation. These strains however often fail to nodulate because they are unable to overcome environmental stresses. The inoculant strains also have to compete with the indigenous rhizobia which are often unable to fix N in symbiosis. In order to compete effectively with indigenous strains, inocula have to be applied in a titer several times higher than the native rhizobia in the soils.

Testing the competitive ability of inocula strain is very important to enhance the effect of inoculation. There are no easy and accurate methods for determining competitiveness of rhizobia in soils and identifying the strains responsible for nodulation. However, with the development of a technique using the resistance of rhizobial strains to antibiotics, strain identification can be carried out without too much difficulty. The method therefore was adopted in this study to select effective and competitive strains of rhizobia for mungbean in marginal soils.

## Methodology:

a. Development of antibiotic resistant strains of rhizobia for mungbean

To investigate the successful establishment and survival of applied rhizobia in the field, the antibiotic resistance marker was employed. This method is very sensitive and reliable in detecting and quantifying the inoculated strains in the nodules of plants.

The intrinsic antibiotic resistance of four rhizobial strain (MCO, CY 44, TAL 441, TAL 209) were developed by exposure of the rhizobial strains to different concentrations of the antibiotics like streptomycin, kanamycin, erythromycin and spectinomycin. Retention or loss of the symbiotic effectiveness of the four (4) antibiotic-resistant rhizobial strains was assayed by using the plant-infection test.

b. Field Experiments (Tanay National Soil and Water Resources Research Center)

Two subsequent field trials were done in Tanay which was previously classified as an Ultisol. The treatments in the experiments were the following:

1. Liming:
    - no lime
    - with lime (3 tons dolomite)

2. Rhizobium strains
    - no inoculation
    - MCO
    - CY 44
    - TAL 209
    - TAL 441
    - Mixture of the strains

The treatments were replicated 3 times and were arranged following the Randomized Complete Block Design (RCBD). In both experiments, the mungbean cultivar planted was PSB Mg-6. Fertilization was based on soil analysis. (29-60-60 kg NPK/ha). At full blooming stage, 10 representative plants from the sampling area in each plot were taken for dry weight determination and nodule occupancy analysis. The plants were grown to maturity for determination of yield and yield components. The general view of the experiment is shown in Figure 1.

Fig. 1. General view of the experiment during the first field trial.

## Salient Findings:

1. All the strains used in the study developed their resistance to antibiotics after three (3) sub-culturing. Result of the confirmatory trial to test retention or loss of infectiveness of the antibiotic resistant strains showed that all the strains retained their infectiveness on their host as shown by the presence of nodules in plants inoculated with the four strains (Fig. 2).
2. For single strain inoculation, strain MCO was the most competitive among the strains tested during the first field trial (82 % nodule occupancy in unlimed soil and 100 % in limed soil) as shown in Table 1. The other strains were not as competitive as strain MCO. However, during the second field trial, recovery of the strains from the nodules was lower. Strain MCO still occupied most of the nodules of plants in soils not amended with lime (75.3 %) while strain TAL 209 occupied 65.4 % of the nodules of plants in soils applied with lime.

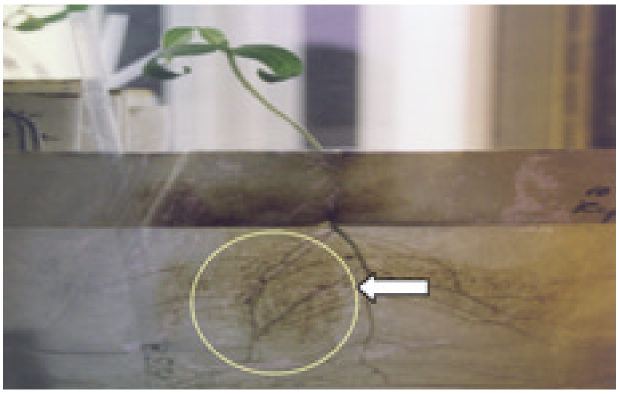

Fig.2. Plant-infection test showing infectiveness of antibiotic-resistant strains for mungbean as indicated by the presence of nodules on the test crop.

In mixed inoculation, the most competitive were strains MCO and CY 44, but the indigenous rhizobia occupied most of the nodules especially during the 2nd field trial (Table 2). This phenomenon showed the very formidable advantage of the indigenous rhizobia in forming nodules. The introduced rhizobia were not able to completely overcome the resident rhi-

Table 1. Percent nodule occupancy of Rhizobium strains in nodules of mungbean plants grown in limed and unlimed soil in Tanay (Ultisol) during the 1st and 2nd field trials.

| Strains | 1st field trial | | 2nd field trial | |
|---|---|---|---|---|
| | Unlimed | Limed | Unlimed | Limed |
| CY 44 | 49.8 | 54.5 | 43.3 | 7.3 |
| MCO | 82.8 | 100.0 | 75.3 | 29.8 |
| TAL 441 | 20.0 | 41.9 | 11.6 | 1.6 |
| TAL209 | 68.4 | 30.1 | 59.3 | 65.4 |

Table 2. Competitiveness of Rhizobium strains in mixed inoculation.

| Strain | 1st field trial | | 2nd field trial | |
|---|---|---|---|---|
| | Unlimed | Limed | Unlimed | Limed |
| Single infection | | | | |
| CY 44 | 2.0 | 0 | 8.3 | 2.3 |
| MCO | 10.5 | 12 | 6.0 | 16.0 |
| TAL 441 | 0 | 0 | 0 | 0 |
| TAL 209 | 0 | 0 | 0 | 10.6 |
| Double Infection | | | | |
| CY 44 + MCO | 30.53 | 24 | 0 | 0 |
| CY 44 + TAL 441 | 0 | 0 | 0 | 0 |
| CY 44 + TAL 209 | 0 | 0 | 3 | 0 |
| MCO + TAL 441 | 0 | 0 | 0 | 0 |
| TAL 441 + TAL 209 | 0 | 0 | 0 | 0 |
| MCO + TAL 209 | 3.5 | 19 | 3 | 8.6 |
| Triple Infection | | | | |
| CY 44 + MCO + TAL 441 | 11.6 | 16.6 | 0 | 7.6 |
| MCO + TAL 441 + TAL 209 | 0 | 1.6 | 0 | 0 |
| CY 44 + TAL 441 + TAL 209 | 0 | 0 | 2.57 | 0 |
| CY 44 + MCO + TAL 209 | 20 | 20 | 0 | 0 |
| Infection of 4 strains | 0 | 0 | 0 | 0 |
| Indigenous rhizobia | 21.87 | 6.8 | 77.13 | 54.9 |
| Total | 100 | 100 | 100 | 100 |

zobia which formed a bulk of the total nodules analyzed.

3. Response of mungbeans to inoculation of the different strains showed that on the average, a slight increase on the seed yield of the plant was observed with inoculation. Liming seemed to have a negative effect on the yield of the plants during the first field trial, however, during the second trial, the application of lime increased the seed yield irrespective of inoculation treatment (Figure 3.)

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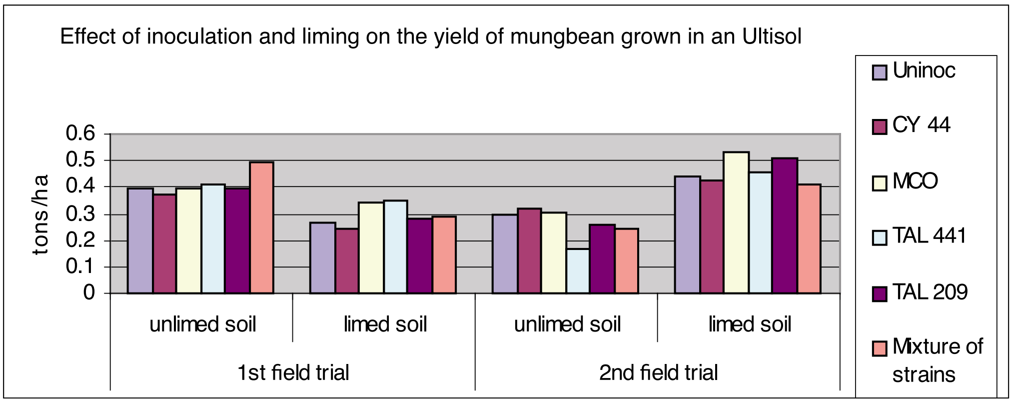
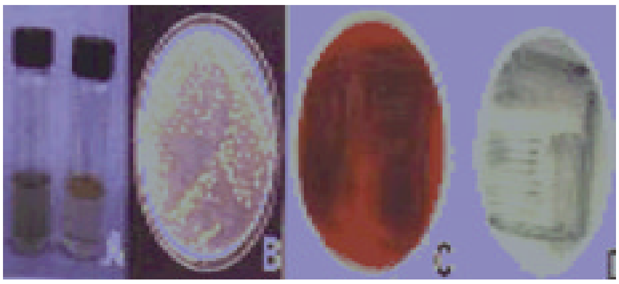



Fig. 3. Inoculation and liming effects on the seed yield of mungbean grown in an Ultisol.

## Philsurin developed Biofertilizer for sugarcane

The Philsurin (Philippine Sugar Research Institute Foundation, Inc.), a non-profit organization created in 1995 has developed biofertilizer for sugarcane in line with its functions to undertake sugarcane research, development, and extension. Sugarcane production requires high fertilizer inputs, thus development of biofertilizer for sugarcane entails reduction of production cost. Sugarcane was found to have the capability of obtaining nitrogen from air for its development through the association established with nitrogen fixing bacteria called as diazotrophic endophytes.

Philsurin microbiologists, Dr. Lucille C. Villegas has conducted a research to develop microbial inoculant for sugarcane by finding superior strains of diazotrophic endophytes to develop viable nitrogen fixing in association with sugarcane. Promising isolates have been selected and identified and the selected strains can be successfully introduced to sugarcane in vitro. Philsurin reported promising results. Inoculation on micro propagated sugarcane indicates that four isolates positively affected the tillering capacity of the plants. There was no significant difference on the number of tillers between the inoculated plants grown at suboptimal level of nitrogen and the plants grown at optimum level of nitrogen indicating that bacterial inoculation can replace needed nitrogen to meet the optimum level of growth.




*Diazotrophic endophytes on different media*
*A) thick pellicle in semisolid N free medium, B) orange colonies on N free agar, C) chocolate brown growth on glucose yeast extract CACO3 agar D) greenish brown grown growth on potato agar.*

Pot experiment was also conducted to evaluate the effects of inoculation at different developmental stage of sugarcane. Seven bacterial isolates were inoculated on four sugarcane varieties (VMC 86-550, VMC 87-599, PS3, and Phil 80-13). Agronomic parameters of sugarcane were evaluated as affected by the inoculation of different bacterial isolates. No result however was reported for this aspect.

*Inoculated plants growing under natural conditions*

<Source>

Dr. Lucille C. Villegas, Ph.D. Development of Microbial Inoculant for Sugarcane. Philsurin Annual Report 2002 - 2003. Pg 39-41.

# BIOTECH Fertilizers: the approach to improve crop productivity and quality

Over at the National Institute of Molecular Biology and Biotechnology (BIOTECH) in the University of the Philippines Los Banos, the mood is upbeat as scientists work their way in producing agricultural biotechnology breakthroughs that would combat global hunger - biofertilizers which they hope would solve the never-ending problem on food security.

Biotechnology, a set of scientific tools that makes use of living organisms, such as microorganisms to generate products and services, has been doing wonders to agriculture and food sector. At BIOTECH, the first wave of products paid off in the form of environment-friendly yet affordable microbial-based biofertilizers and inoculants that can substantially double the yield of rice, corn and other important crops and substitute for chemical-based farm inputs. These products, generated by the Biotechnology for Agriculture and Forestry Program, underwent a series of trials - from greenhouse to larger field testing - to determine if they are ready for commercialization and for transfer to private entrepreneurs. These are classified as: biological nitrogen fixers, mycorrhiza, plant growth promoters and microorganisms that can degrade organic wastes.

## Biological nitrogen fixers

The use of microorganisms to harness elemental nitrogen through nitrogen fixation is one of the promising alternative technologies to address the high cost of chemical nitrogen fertilizers and the increasing environmental problems attributed to their use. These microbes, known as biological nitrogen fixers (BNF), can directly fix nitrogen gas from the atmosphere to make it usable by the plants.

An example of this microorganism is the rhizobia, which reside inside the nodules of leguminous plants. The mixture of rhizobia, sterile soil and charcoal led to the development of an inoculant known as **NitroPlus**. It is a biofertilizer specific for soybean, mungbean, cowpea, peanut, garden pea, pole sitao and other food legumes. NitroPlus comes in powder form in 100-gram packet which is used to coat seeds before sowing. One hectare requires 4-5 packets which is a lot cheaper than 2-3 bags of ammonium sulfate. Field trials done in collaboration with various agencies showed an increase in soybean yield by 124%, mungbean by 29% and peanut by 39%. It was also found to replace 30-50% of the nitrogen requirements of plants and has a fertilizing value equal to 30 kg N/ha. Presently, NitroPlus is being tested on non-legumes.

BIOTECH is also successful in producing **BIO-N**, a biofertilizer that contains *Azospirillum*, a bacterium isolated from the grass *Saccharum spontaneum* or talahib. BIO-N enhances root development, growth and yield of plants (rice, corn and other agricultural crops) and provides 30-50% of their nitrogen requirements. Efficacy testing of Bio-N on rice variety RC 18 showed improvement in the average number of tillers, straw and grain yields. Five packs of Bio-N are needed to inoculate a hectare of rice or corn. It was also found to increase yield of vegetables (tomatoes, okra, eggplant, lettuce and ampalaya). BIO-N is available in powder form in 200-g pack, which is meant either for seed inoculation, direct broadcasting over seeds or mixed with water as root dip.

## Plant Growth Promoters

Some microorganisms produce plant growth regulators such as auxins, gibberilins and cytokinins which can be used in plant propagation.

**BioGroe** is a biological fertilizer containing plant growth-promoting bacteria (PGPB) that influence root growth by producing plant hormones and provide nutrients in soluble form. It is packaged as a powdered inoculant that can be used for almost all types of plants - vegetables, ornamentals, flowering plants and herbs. Experiments done at BIOTECH showed that it increased *ampalaya* yield by 90% and lettuce by 40%. *Dendrobium*, on the other hand, had early flowering while *Mussaenda* and *Hibiscus* (gumamela) produced heavier and longer roots. It was also found effective in the rooting of blackpepper cuttings and hard-to-root ornamentals *Eugenia* and *Bushida*. BioGroe also promoted leaf bud formation and plant survival of coffee stem cuttings and enhanced seed germination of the herbs marjoram and thyme. In the efficacy test conducted in Tarlac and Cavite, BioGroe was found to enhance seedling growth and lowered farmers' production cost.

**Cocogro** is a mixture of plant growth hormones derived from coconut water. This natural product can be used as a fertilizer supplement to enhance and promote growth and yield of plants, especially tissue cultured orchids and orchid seedlings. Researchers have proven that orchid seedlings, when sprayed with Cocogro, increased in growth by 100% within a month. Field trials showed that Cocogro can increase yield of beans by 64%, peanuts by 15%, sweet pepper by 100% and Chinese cabbage and by 20-30%. Cocogro is also good for ornamentals, cereal crops, fruit trees and flowering plants. It increases root proliferation, shoot development, bud formation and early flowering.

## Mycorrhiza

Certain microorganisms also provide phosphorus and other nutrients. Mycorrhiza is a symbiotic association between the roots of plants and a fungus. The association provides many benefits to plants such as increased absorption of nutrients and water. It likewise serves as a biological control agent against root infection and can improve soil properties. BIOTECH has produced four mycorrhizal products: Mycogroe, Mykovam, VAM Root Inoculant and BrownMagic.

**Mycogroe** is a biofertilizer in tablet form containing spores of ectomycorrhizal fungi. The fungi, when inoculated to reforestation seedlings, will infect the roots and help absorb water and nutrients particularly phosphorus. They can also prevent root infection by pathogens and increase plant tolerance to drought and heavy metals. Mycogroe was found effective in promoting the survival and growth of trees and can replace 60-85% of their fertilizer requirement. Application is done only once throughout the entire life of the tree. Mycogroe is applicable only for trees such as pines, dipterocarps, *Eucalyptus*, *Alnus*, *Acacia* and *Casuarina*. One tablet is inoculated into each seedling during transplanting in the nursery.

On the other hand, **Mykovam** is a powdered biofertilizer which has effective species of vesicular arbuscular mycorrhizal (VAM) fungi. Basically, its functions are similar to that of Mycogroe. The fungi infect the roots, form a close association with the plant and assist in absorbing water and nutrients such as phosphorus, zinc and nitrogen. Mykovam can be applied to almost all plants: agricultural crops (upland rice, corn, tomato, eggplant, onion, garlic, pepper, cassava, and sweet potato), fruit trees (guava, rambutan, papaya, citrus, banana, lanzones, coffee, guyabano, coconut and mango), ornamentals and reforestation species (acacia, gmelina and mahogany). Mykovam even fights destructive pests and diseases and increases plants tolerance to drought and heavy metals. Use of this product reduces 60 -85% of the fertilizer requirements of most plants. It is economical to use since a kilo of Mykovam can fertilize 400 seedlings.

**Brown Magic** consists of finely chopped and powdered fruiting bodies of endomycorrhizal fungi. It is especially effective for *in-vitro* cultured orchids and provides the nutrients necessary for their growth. It also serves as a biological control agent of root infection and other harmful pests and diseases. Experiments done at BIOTECH showed healthier, greener and more vigorous *Dendrobium*, *Vanda* and *Cattleya* seedlings. Plants had more suckers and spikes and produced flowers earlier and at a longer period of time.

**VAM Root Inoculant** is composed of dried chopped roots infected with VAM fungi. These fungi assist plant roots in absorbing water and nutrients and thus reduces chemical fertilizer requirement of crops. VAM Root Inoculant also acts as biocontrol agent against root pathogens and produces certain substances that promote plant growth and improve soil structure. Experiments done at BIOTECH showed improved growth and fruiting capacity of papaya, increased yield of peanut and enhanced phosphorus uptake of bitter gourd. It is also an effective growth promoter of agricultural (banana, sugarcane, corn, root crops) and horticultural crops, vegetables (eggplant, okra, tomatoes, onion), fruit trees and ornamentals.

## Microorganisms that decompose agricultural residues

BIOTECH has produced an organic fertilizer technology through composting which can significantly enhance crop growth and yield. Chicken, swine or cattle manure can be combined with farm wastes such as rice straw, coffee hulls and corn stover or with agro-industrial wastes such as sugarcane bagasse and mudpress and processed into bio-organic fertilizer. The technology also involves the use of two inoculants: **Bio-Quick** which contains *Trichoderma* sp. that can degrade organic waste materials in only 3 to 4 weeks instead of almost 6 weeks, and **Bio-Fix,** the enrichment inocula containing *Azotobacter* sp. which is added to the compost heap to increase the nitrogen content of the final product. The end-product called Bio-Green can be applied to all kinds of plants and can increase yield by 20-30%. It also serves as soil conditioner, enhances the nutrient uptake of plants in soil and thus makes plant healthier. **Bio-Green** can be applied basally or directly depending on the crop. If applied at 10 to 20 bags/ha, it can equal the effects of chemical fertilizer at 60-30-30.

## Promotion and Technology Transfer of BIOTECH Biofertilizers

Research does not end with the discovery of a technology or process. More important is the utilization of the technology or process by intended clients. Thus, at BIOTECH, researchers are not just keen on producing high quality fertilizers but also in reaching the farmers, even those in marginal areas of the country. They go beyond the confines of laboratories to generate and sustain a high level of awareness and acceptance of biofertilizers. They do these in tandem with the extension and communication specialists of the Institute. One promotional approach used is the production of an integrated information package through print and multimedia materials. Biofertilizer fact sheets or leaflets are given to farmers and extension workers as supplementary materials during seminars, forums or trainings, where researchers serve as resource speakers. Another approach is to facilitate institutional collaboration and establish links with different agencies, including local government units, in the transfer or commercialization of BIOTECH biofertilizers. They also collaborate with setting-up field demonstration areas to see the efficacy of our biofertilizers.

Case in point: to create awareness and encourage extensive support and patronage of farmers, the Bio-N staff work hand in hand with the Department of Agriculture in implementing the transfer of the technology in three different phases. Training workshops on Bio-N application were held in different regions of the country complemented by presentations, posters, handouts and product samples for actual testing. Bio-N mixing plants were likewise established to address the problem on the unavailability of supply and/or accessibility to distribution centers. BIOTECH also partners with the Technology and Livelihood Resource Center in marketing Bio-N.

However, Bio-N is just one of the biofertilizers which is being aggressively promoted by the Institute. Media exposure for NitroPlus, Mykovam, Mycogroe, BrownMagic and BioGroe were also sought to intensify our information campaign on biofertilizers. As a result, researchers were interviewed in various radio and television programs while feature articles of their technologies were published in newspapers, magazines and newsletters. Demonstration trials done both at BIOTECH and in farmers' field validated the efficacy of the biofertilizers, much to the satisfaction of farmers.

To facilitate the efficient technology transfer from the laboratory to target industries and/or beneficiaries, BIOTECH prepares pre-feasibility studies, industry analyses and business plans. Applications for experimental use permit, product registration, technology licensing, joint venture are likewise done. Bio-N for rice and NitroPlus for soybeans are now registered with the Fertilizer and Pesticide Authority, as a result of our techno-demo farms utilizing the idle lands around BIOTECH.

Judging from the increasing demand from farmers and plantation owners, BIOTECH is indeed fulfilling its mission of developing fertilizers that do more than just provide more bountiful yield. The plethora of interest generated by our technologies inspires our researchers to continue the research, development and extension programs they have started. Concomitantly, they hope to further widen the partnership with different public and private agencies, biotech firms in particular, to facilitate the commercialization process of BIOTECH biofertilizers to make them readily available to farmers in different regions of the Philippines and in other countries as well. (**Imelda V. Garcia**, *Program Leader, Communication and Technology Utilization Program of Biotech*)

# *VITAL N*^R : A Revolutionary Biofertilizer Technology with a Fast-growing Market

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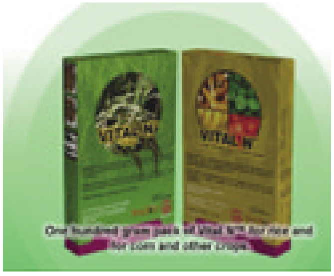


A relatively young but revolutionary biotech product such as ***VITAL N***^R is now getting a lot of attention. The demand for cost-effective and innovative ***VITAL N***^R biofertilizer is rapidly growing. In fact, ***VITAL N***^R now has a fast-growing list of distributors/dealers all over the country.

***VITAL N***^R is a wettable powder formulation that is available in a very convenient 100-gram pack. This small package packs a lot. It acts as a virtual nitrogen fertilizer factory at the roots of the plants. It protects plants from root and leaf diseases. It also makes plants drought-resistant. Not only is it convenient and powerful, it is also easy to store. It can be stored at room temperature for at least 3 years.

***VITAL N***^R is a breakthrough and sustainable agriculture technology for increasing farmers' incomes and food sufficiency. Furthermore, it is proudly 100% Filipino! The product is the result of my years of research together with my scientist wife, Dr. Saturnina C. Halos. Together with our children and in-laws, we formed a progressive biotech company, Arnichem Corporation, to make the product available to the average Filipino farmer. With this product, we hope to re-***VITAL***ize the nation.

***VITAL N***^R is an FPA-registered organic biofertilizer that replaces as much as half of the recommended fertilizer. This translates to a savings of 4 bags of fertilizer per hectare (ha) or about P3,000 for rice and corn and 6-8 bags of fertilizer/ha (about P5-6,000/ha savings) for vegetables. It also increases farmer's profitability by at least 15%, and in some cases more than 100%. Tests conducted by independent scientists and farmers in rice and corn show that, ***VITAL N***^R increases yields by at least one ton per hectare. In onion, additional yields of 15 tons per hectare can be realized by using the product. With its efficacy and the low cost of the product, it is well suited for the majority of small farmers who can not afford the full cost of recommended fertilizer requirement and who badly need a harvest booster.

The following tables (Tables 1-3) were part of the technical data obtained by Dr. Nora

Table 1. The effect of ***VITAL N***^R inoculation in OPV corn (white corn) yields

| Fertilization | Yield (T/Ha) | Added yield(T/Ha) due to ***VITAL N***^R |
|---|---|---|
| No added fertilizer | 4.325 | |
| Plus ***VITAL N***^R | 5.583 | 1.258 |
| ½ RIF | 5.842 | |
| ½ RIF+ ***VITAL N***^R | 7.283 | 1.441 |
| Full RIF | 7.825 | |
| Full RIF+ ***VITAL N***^R | 9.092 | 1.267 |

Table 2. The effect of ***VITAL N***^R on yellow hybrid corn (DK818Y) under late drought conditions.

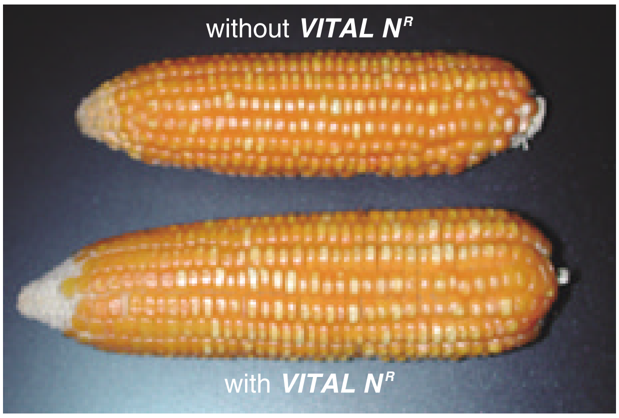
| Fertilizer treatment | Yield (T/Ha) | Added yield due to ***VITAL N***^R(T/Ha) |
|---|---|---|
| No added fertilizer | 2.823 | |
| Plus ***VITAL N***^R | 5.427 | 2.604 |
| ½ RIF* | 4.613 | |
| ½ RIF+ ***VITAL N***^R | 7.595 | 2.982 |
| Full RIF | 6.516 | |
| Full RIF+ ***VITAL N***^R | 9.049 | 2.533 |

Table 3. The effect of ***VITAL N***$^R$ on rice yields

| Fertilization | Yield (T/Ha) | Additional yield (T/Ha)due to ***VITAL N***$^R$ |
|---|---|---|
| No fertilizer applied | 3.40 | |
| Plus ***VITAL N***$^R$ | 5.02 | 1.62 |
| 1/2 RIF* | 5.52 | |
| 1/2 RIF+ ***VITAL N***$^R$ | 6.34 | 0.82 |
| Full RIF | 6.29 | |
| Full RIF+ ***VITAL N***$^R$ | 7.25 | 0.96 |


Kinchay


rice




Corn

Inciong, Consultant, Bureau of Soils and Water Management, Dept. of Agriculture submitted to the Fertilizer and Pesticide Authority in support of the registration of ***VITAL N***$^R$ as a biofertilizer. (RIF-Recommended Inorganic Fertilization rate).

The following are results obtained by undergraduate thesis students of Dr. Betty Malab, Professor of Soil Science, College of Agriculture, Mariano Marcos University, Batac, Ilocos Norte. The results show that 1/2 RIF plus VITAL NR give similar yields as those of RIF.

Farmer scientists participating in the program of Dr. Romulo Davide in Bantayan, Cebu obtained more than 100% increase in yields when they applied ***VITAL N***$^R$ (3.86 MT/ha) compared to their practice of applying nothing (1.76 MT/ha). This yield was comparable to what they obtained when they applied complete fertilizer (14-14-14) also at 3.86MT/ha. In Toledo City, using IPB 929, an increase of 62.5% of corn treated with ***VITAL N***$^R$ (3.77 MT/ha) was obtained over that without any treatment (2.32MT/ha).

Table 4. The yield of pepper (*Capsicum anuum* L) as affected by varying levels of fertilizer and ***VITAL N***$^R$

| **Fertilizer treatment** | **Sweet pepper*** | | **Bontoc pepper**** | |
|---|---|---|---|---|
| Fertilizer treatment | Yield T/ha* | Kg fruit /Napplied* | Yield T/ha** | Kg fruit /N applied** |
| No added fertilizer | 2.55[b] | - | 2.26[e] | - |
| 140-50-50 (Full RIF) | 6.58[a] | 51.16 | 8.19[ab] | 42.37[c] |
| 70-25-25 (1/2 RIF)+ ***VITAL N***$^R$ | 4.09[a] | 46.94 | 6.60[b] | 61.98[c] |
| 35-12.5-12.5 + ***VITAL N***$^R$ | 3.76[ab] | 29.55 | 5.80[bc] | 101.22[a] |

* From Tabora ML Jr Unpublished Thesis, MMSU 2005
**From De Peralta GC Unpublished Thesis, MMSU 2005

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**Research Interest**

- □ Use of nuclear techniques in agricultural research
  - ● Use of 15N isotope dilution technique in fertilizer use efficiency and biological nitrogen fixation studies
  - ● Use of fallout radionuclide 137Cs in soil erosion and sedimentation studies

**Positions held**

- □ Project Leader, Forum for Nuclear Cooperation in Asia Biofertilizer Project
- □ Project Leader, Department of Agriculture-Bureau of Agricultural Research funded project on the Use of Cs-137 in Estimating Soil Erosion and Sedimentation
- □ Member of the National Project Team for International Atomic Energy Agency RAS/5/043 project on Sustainable Land Use Management and Strategies for Controlling Soil Erosion and Improving Soil and Water Quality

**Check FNCA Web Site!**
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