THE DEVELOPMENT OF NON-SYMBIOTIC NITROGEN FIXING INOCULANTS FOR RICE PRODUCTION IN THE PHILIPPINES: A COUNTRY REPORT /1

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INTRODUCTION

The population in the Philippines has been increasing through the years, but crop production remains relatively low per unit area. The agricultural lands, especially those along the high ways are converted into industrial centers and housing subdivisions. As a consequence, there is a reduction in effective agricultural land area to be planted to crops and a concomitant insufficient produce for the growing population. Continuous harvesting from the small area can result to decline productivity and harvests due to the depleted conditions in the soil nutrients. The currently used agricultural inputs are mostly chemical in nature and imported. For example the inorganic nitrogenous fertilizer sources are 100% imported, thus their prices have been escalating to an extent that the farmers cannot afford to apply the recommended fertilizer rates for their crops. The use of chemical fertilizers has been widely practiced because of its almost immediate effect, this has somehow replaced the traditional way of recycling the nutrients to a point that this is now taken for granted. The country that used to be self-sufficient in rice has have an efficient and sustainable crop production on the remaining agricultural land areas has been felt through the years. It is now the thrust of the government to integrate inorganic and organic fertilizer management in addition to the already popular integrated pest management.

SCOPE OF THE REPORT

The report covers only the progress and problems in the search for beneficial nitrogen fixing bacteria that benefits rice. There are other available data for corn and sugar cane but due to time constraint, the presentor was not able to gather all the secondary data from research records of the institute. The paper tries to present the potential of the bacterial isolates being developed into a promising inoculant for rice.

SOME REALITIES

It is known fact that plant growth is the result of the interaction of genotype and the environment to which is and will always be the source of the elements for plant growth and yield. Continuous harvested of the elements through crop removal without replenishment leads to a depleted soil condition. The application of solid inorganic fertilizers such as NPK (14-14-14), urea (46-0-0), ammophos (45-60-0) and some other chemical combinations is not enough to replace the harvested nutrients. Plants need a complete array of macro-elements and micro-elements for growth and yield. In fact some 16 elements are already recognized to be vital in the synthesis of biomass by plants. Of these elements, the 13 are not commonly contained or supplied by the common solid inorganic fertilizers. These are usually commonly derived from the soil while the other

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3 are obtained free from the atmosphere. These 3 elements are nitrogen, oxygen and carbon. Nitrogen, when in the gaseous form (N_2), is not available for plant use and in order to be converted to available form it needs to be fixed through either the industrial process (Haber Bosh Process) or through biological nitrogen fixation (BNF).

Nitrogen is the most critical element among the 13 elements because it is almost always subject to leaching, volatilization and denitrification. The Haber Bosh Process makes use of fossil fuel or petrol while BNF is made possible through the action of the nitrogenase enzyme present in the bodies of certain species of bacteria and blue green algae. Some species of nitrogen fixing bacteria are free-living in the soil and dependent on organic matter for their energy while the others are either associated loosely with root tissues of plants such as *Azopirillum* or in symbiosis with plant roots as in the Rhizobium-legume system.

THE INSTITUTE OF MOLECULAR BIOLOGY AND BIOTECHNOLOGY

The National Institute of Biotechnology and Applied Microbiology (now known as the National Institute of Molecular Biology and Biotechnology) or BIOTECH was established in 1979. One of its mandate was the search for alternative fertilizer technologies that shall enhance and sustain crop production. During that time and even in the present, the country imports inorganic fertilizers the bulk of which are composed of nitrogenous forms.

TECHNICAL BACKGROUND

In the late 70s one of the projects in the institute focused on non-symbiotic nitrogen fixation or associative symbiosis by non-legumes. Surveys for root-associated bacteria were started from grasslands in Luzon island through isolation of nitrogen fixers using nitrogenase assays and subsequent purification and screening for effectiveness against rice and corn. After 5 years of continuous screening using *in vitro* and *in situ* methods under laboratory condition, 3 strains were identified to be high N-fixing. One of the bacterium was derived from the roots of rice (*Oryza sativa L.*) and 2 from the roots talahib (*Saccharum spontaneumL.*). These were further tested in pot cultures using rice and corn as the test plants.

The bacteria were found to be adsorbed to rice root hairs within minutes in the presence or absence of combined nitrogen in the growing medium, however significantly, more cells were attached to root hairs of rice seedlings which were grown in the N medium which reached a peak at 6 hours and leveled off at 24 and 48 hours. The introduction of the bacteria to rice seedlings through root dipping increased uptake of NH_4-nitrogen, phosphorus and potassium as well as biomass of one week old test plants (Santos & Garcia, 1992).

Initial inoculation tests with the isolates Bos 179 and BSs 202 on potted dry land rice variety UPLRi-5 grown in Maahas clay either in the absence or presence of 50 and 100 kg N/ha rates, exhibited significantly taller test plants and with more dry matter than the uninoculated ones (Tables 1). Several other screening tests using the two isolates simulating both dryland and paddy conditions suggested that the grain yield of inoculated plants were comparable with those that were fertilized with the recommended nitrogen fertilizer rates for the particular test host (Umali-Garcia et al, 1989).

THE INOCULANT

The inoculant that was used in the field trials is in a solid from, the carrier of which is a combination of sterile soil and charcoal. The particular inoculant is currently named BIO-N. BIO-N comes in a 200g packet and 4-5 packets are recommended to inoculate seeds or seedlings enough to plant a hectare. The current version of the inoculant needs improvement in terms of quality and storability.

RESEARCHER-MANAGED TRIAL:

The first field test using the *S. spontaneum* isolate and the rice variety, UPLRi-5 was conducted in 1989 in Calaca, Batangas under dryland condition in a Taal sandy loam soil. A mean increase of 88% in the number of filled grains per panicle of the inoculated plants over the uninoculated counterpart was detected. This agronomic character was reflected in the grain yield of inoculated plots with an increase of 91% over the uninoculated plants. In all cases, the inoculated plants were observed to be more vigorous and had greener leaves than the unfertilized and fertilized uninoculated controls especially during the second and third months of growth (Table 2). In central Philippines, several upland rice farmers have also used the inoculants in upland conditions and have claimed that the yield have been improved by bacterization.

Another field trial on the effect of the two bacterial isolate, Bos 179 and BSs 202 was conducted on two IRRI rice varieties, IR 64 and IR 52, at the International Institute of Rural Reconstruction (IIRR) in Silang, Cavite in the same year in the collaboration with an American Peace Corp volunteer. In the particular on-farm test only a minimal N-fertilizer input of 24 kg N/ha rate was used. The two IRRI rice varieties can produce an average of 2.1 tons when fertilized with only 24 kg N/ha rate but the yield of the two varieties could be doubled by bacterization. The yield of rice variety IR 52 was increased to 86.3% and 100% when inoculated with Bos 179 and BSs 202, respectively, in contrast to that of IR 64 which could exhibit only 30.4% increase in grain yield in the presence of Bos 179 and 91.3% with BSs202. It was further noted that suggested that the interaction between the bacterial isolate and rice genotype is an important factor to consider in inoculation tests.

Another on-farm test was conducted by the BIOTECH staff in the same locality but in different field sites using a different paddy rice variety, IR 72. The N fertilizer rates were varied from zero, 20, 40 and 60 kg/ha and with a constant rates of 40 kg P/ha for all plots. There was no addition of K as there was sufficient potassium in the soil.

Plant height of inoculated plants was significantly taller in the 20-40 kg N plots but was statistically similar to the uninoculated counterpart with 40-60 kg N rates. The mean number of tillers per hill was not different among inoculated treatments but inoculation increased tiller production b 48%. The grain yield of all inoculated treatments in the absence and/or presence of combined N were statistically the same with an average of 6.2 tons to a hectare. The average yield of 5.4 tons per hectare was observed from the fertilized uninoculated plots but this was shown to be statistically the same with 4.8 tons from the unfertilized plots (Table 4) suggesting that the farmer's field was fertile enough to yield more than the national average of 2.3 for rice. Inoculation improved grain yield by 14.8% which was equivalent to 0.800 kilograms or 18 sacks of palay (Table 4).

FARMER MANAGED TRIALS

In the WS 1993, verification trials using the BSs 202 inoculant were conducted in three sites in Luzon (Table 5). The rice varieties that were used differed with site based on the preference by the farmer cooperators and rates of NPK fertilizers used for the particular farm. The purpose of the said trial was to demonstrate to the farmers that inoculation in rice could improve yield and minimize inorganic N input. In this test, the farmers were left to attend to their field and the only time that the researchers were physically present was during the layout of the trial and at harvest time.

The results obtained from such trials were not as consistent as in the researcher managed tests as some of the data from some treatments were not collected because the farmers harvested the plots ahead of scheduled date (Santos, et al. 1991). A trial in Parian, Calamba, Laguna using C1 rice variety suggested that BIO-N inoculation alone could yield 5 tons while inoculation in the presence of 22.5 kg N/ha rate gave only 4.0 tons. A very much lower yield of 3.0 was obtained from the plots fertilized by 45 kg N/ha (Table 5).

In Camarines Norte where the yield of rice was lower than the national average, inoculation and/or fertilization with 45 kg N/ha produced comparable yield of 1.4 tons/ha using a traditional 75-day variety. This would suggest either a complete substitution of half of the recommended rate or a satisfaction of the needs of the variety for the element nitrogen (Table %). The lower yield in this test site may be more inherent in the variety used as this was an early maturing variety but may also be attributed to the marginal soil characteristics in the site. The soil in the site in Camarines Norte had a pH of 4.5, an organic matter level of 2.3, 0.13% N, 3.5 ppm P and sufficient potassium.

In the Los Banos site, an average yield of 3.30 tons were recorded from both the plants inoculated with BIO-N + 45 kg N/ha and the uninoculated but fertilized with the recommended rate of 90 kg N/ha suggesting a substitution capacity for 50% of the full fertilizer requirement of the test variety. It should be noted that the tests in Los Banos and Labo did not exhibit significant differences among treatments, however, if the basis for comparison shall be the treatment that involved inorganic N, then it can be deduced that the use of BIO-N can still be advantageous in terms of fertilizer expense.

During the past decade the initial intention b the University administrators was to transfer the technology to any qualified business agency and allow them to develop the technology with sufficient technical support from BIOTECH. Nevertheless, occasional information flow on the availability of the inoculants had reached the which contributed to the increasing awareness on the use of this biological fertilizer. This could be gleaned from the may request for farmers seminar and forum. The problem on the availability of the inoculant to farmers in the different regions of the county is already recognized as shown b the clamor for the inoculants by farmers is no, longer considered academic but a real one.

A Biotechnology Village was piloted b BIOTECH in the town of Infanta, province of Quezon in Luzon island using the BIO-N and several other technologies of the institute in 1997-98. Seven out 10 farmers claimed satisfaction over the use of BIO-N (Tables 6 & 7). The results from the Infanta farmers' tests cannot be compared with the uninoculated treatments, as the farmers did not want to risk the yield that they would get from untreated plots. The inoculant was only used in addition to the farmers current fertilization schemes.

Farmer-interviews conducted lately in Jala-jala, Rizal and in Laguna provinces suggest that the farmers were willing to continue with the use of the technology not only because of the increase ield and less N-fertilizer input due to inoculation but also because of the obvious less pest and disease incidence. One farmer scientist also claimed protection of directly seeded rice seeds from being picked up by birds because inoculated rice seeds are not noticed easily. The stems of inoculated rice are studier and harder than the uninoculated N-fertilized plants.

DISCUSSION

The earlier tests were conducted primarily to assess the benefits from bacterization or inoculation based on the ability of the isolates to form an intimate association with grass host, fix atmospheric nitrogen and improved root development. As reflected from more controlled experiments, some kind of complementation with inorganic fertilizer amendment has been achieved especially in poor soils. Statistics suggest yield inconsistencies especially those obtained from the farmers' field. This made it difficult to interpret the results, as there was no single quantitative data to suggest a inconsistent large yield gaps among farms and varieties may be attributed to any of the following factors:

1) heterogeneity of the field sites;
2) the treatments that were used were not the same for all field sites;
3) yield potential of the different varieties;
4) the cultural practices given by the farmer to the test plant;
5) method of growing the test host and the timing of introduction of the bacteria; and
6) the failure of the farmers to follow the directions on the use of inoculant.

Some farmers would introduce the inoculant as seed inoculant, dusting the two day old germinants or used as a root dip or used in watering the seedbed. There has been no studies that tried to compare the effect of the method of inoculation on the response of the rice crop.

The trials used different varieties in different field sites. The use of the different varieties in the same field site may demonstrate interaction of genotype and inoculant strains.

The role of rice variety contributes a lot to yield. In the farmers field conducted under the auspices of IIRR in Cavite, the interaction of the inoculant and the variety was demonstrated. In Luzon, while inoculation was suggested to complement 50% of the N-input 3 sites, the yields obtained were lower than the national average because of the obvious effect of variety on the overall results of the trials e.g. a short maturing variety can be expected to produce lower yield than the longer maturing ones. Also the short maturing variet when grown on soil of marginal fertility can also be expected to produce lesser grain yield than when the one grown in a rich soil.

It is also important that cultural practices, type of fertilizer and rates for this matter should be standardized such that results ma be attributed to the effect of inoculation.

The farmers in the Philippines were found to differ in methods of cultivating rice especially in staring the seedlings. The method and timing of introducing the inoculant differed with regions and cultural group.

Other difficulties that were encountered in the use of rice inoculant is inconsistency in growth parameters. Factors that limit migration of beneficial bacteria in the soil, adsorption to soil particles and possible competition with the natural populations of rhizospheric bacteria for nutrients leaking from the roots may differ with isolates and soil. The maintenance of the bacterial population or its decrease can affect the positive effects on plant growth. Hence, monitoring and evaluation of population dynamics of soil microorganisms becomes a necessary tool in assessing the effects of inoculation on the test host plant.

The competitive ability of *Azopirillum* as determined by root surface area changes, is diminished by organic matter once its concentration in the soil becomes greater than 1% because other bacterial population can reach 10^7 to 10^8 cfu/g soil and which offers a strong competition with the bacterial inoculant (Fallik and Okon(1988). It is also possible that the concentration of minerals and nutrients could provide sufficient nutritional sources for both the test plant and the symbiont such that the associative interaction is lessen or broken down. The effects of more than 1% organic matter content in the soil have been suggested in the yield obtained from both the researcher-managed trials and the farmers reported previously. Except for one of the researcher-managed trials reported in this paper, most of the soil have 2% or more organic matter content. Nine out of the 10 pilot farms under the biotechnology Village Project may have contributed to the inconsistencies in the yield derived from the farmers field. It is important that the effects of inoculation on yield and yield parameters as influenced by each of the soil factors and/or their interactions are quantified.

While there is obvious positive effect of inoculation on growth of host plant, nutrient balance studies have not been conducted, so much so with quantification of the contribution of the inoculant to the nitrogen economy of the plant. The use of nuclear techniques (^{15}N isotope) may be an important tool to assess the contribution of the inoculant strain on the rice crop. Also, in the preparation of the inoculant i.e. irradiation of carrier/substrate should be considered.

The inoculant technology for rice and corn has been recognized by PCARRD as earl as 1989, but the development of the inoculant has been going on a tortoise phase. It is only recently that attention in the form of establishment of techno-demos has been extended by the Department of Agriculture, the most government agency. With increasing demand by walk-in farmers and farmer-cooperatives from the different regions of the country, the status in the use of the BIO-N may no longer be considered academic but real. There is a real demand for an alternative fertilizer technology.

After five ears of information efforts made by the staff of BIOTECH, the Department of Agriculture finally recognized the technology through a grant that meant to popularize the use of BIO-N not only for rice and corn but to test it on high value crops. While the inoculant does not exhibit specificity, the effectiveness of which has not been thoroughly tested for high value crops.

The Technology Livelihood Resources Center has recognized the importance of continuous supply of the product through a funding for the establishment of small structure to house the production. However, there is need to improve the quality and storage life of the inoculant for a broader and wider distribution and use.

In spite of the yield increases reported and as claimed n the farmers, the effects of inoculation on yield and agronomic characters have been inconsistent. It is obvious that there are research gaps in both the basic and applied aspects in the development of the inoculants. The following may help minimize the problems associated with the utilization of rice inoculant.

RECOMMENDATION

On the basic aspect, there is need to understand the role and contribution of the particular bacteria contained in the inoculant. Where and whenever necessary, a nitrogen or nutrient balance studies should be conducted on rice inoculation tests. It is best to include all possible treatments such as the different levels of N-fertilizer based on the soil analysis and in the presence or absence of organic amendments and of the inoculant to allow comparative and correlation analyses.

There is a need to conduct screening for the most responsive rice variety to inoculation and low nitrogen input if the technology would be developed to support subsistence rice farming.

On the extension aspect, it may be necessary to prepare a readily available and well balanced enumeration sheet especially for farmers who insist on using their own schemes for the purposes of getting information on their previous yield data. When advocating the transfer of the technology, it is important to consider a packaged program involving a holistic approach that shall include not only the technical and agronomic but also the social acceptability and economic aspects of the technology.

Table 1. Interaction effects of three levels of nitrogen and S. *spontaneum*(BSs202) and *Oryza sativa* (Bos 179) root isolates on biomass yield of potted dry land rice variety UPLRi-5. WS 1988.

TREATMENT/ N LEVEL/HA	PLANT HEIGHT (cm)	SHOOT BIOMASS^{ns} (grams)	ROOT BIOMASS (grams)
Control(no fertilizer)	96 .6 bcd	13.82	3.1 c
50 kg N	122.0 ab	18.70 (35.31)	5.2 ab (65.7)
100 kg N	123.5 a	19.15 (38.56)	6.4 a (103.8)
BS s 202, zero N	91.9 cd	13.50 -	4.5 ab (43.5)
BSs 202+50 kg N	96.5 bcd	15.20(9.98)	4.3 ab (38.1)
BSs 202+100 kg N	132.8 a	18.65 (34.94)	4.7 ab (49.8)
Bos179 (no fertilizer)	116.5 abc	19.02(34.73)	4.2 ab (31.7)
Bos 179+50 kg N	86.0 d	14.58 (5.57)	4.0 bc (23.2)
Bos 179+100 kg N	111.8 abcd	19.92 (44.14)	5.6 ab (77.77)

Figures in ( ) are % increases over unfertilized control.

Table 2. Mean effect of inoculation with *S. spontneum* isolate, regardless of fertilizer application, on the yield of dryland rice variety, UPLRi-5 in Taal sandy loam. WS 1989.

TREATMENT	PANICLE LENGTH (cm)	NUMBER OF FILLED GRAIN (per panicle)	GRAIN YIELD (Ton/HA)
UNINOCULATED	21.0 a	74.0 b	2.1 b
INOCULATED	24.1 a	139.0 a	4.0 a
%INC./UNINOC	14.8	88.0	91.0

Means followed by the same letter in the column are not significantly different at 5% level.

Table 3. Effect of inoculation on yield of two wetland IRRI rice varieties, IR 52 and IR 64 in the presence of 24 kg N/ha. DS 1987. General Trias, Cavite.

TREATMENT/ INOCULATION	YIELD (tons/ha)			
	IR52		IR64	
INOC. Bos 179	4.1 a	(86.3%)	3.0 ab	(30.4%)
INOC. BSs 202	4.4 a	(100.0%0	4.4 a	(91.3%)
UNINOCULATED	2.2 b	-	2.3 b	-

Means followed by the same letter in column are not significant different by DMRT at 5% level by DMRT

Table 4. Height, tiller count and grain yield of IR 72 as affected by inorganic fertilizer levels and inoculation with BIO-N in Guadulupe clay, General Trias, Cavite.

TREATMENT	UNINOCULATED			INOCULATED		
N – P – K (Rates kg/ha)	HEIGHT (cm)	TILLER Count	GRAIN YIELD (Tons/ha)	HEIGHT (cm)	TILLER Count	GRAIN YIELD (Tons/ha)
0-40-0	99.8c	11.3c	4.8c	105.0b	17.0ab	6.0ab
20-40-0	99.6c	11.3c	5.7a	109.2a	16.4ab	6.0ab
40-40-0	100.5bc	14.2ab	5.3ab	109.5a	16.2ab	6.6a
60-40-0	106.1ab	14.6a	5.7a	109.8a	17.6a	6.1ab

Means followed by a common letter in the row for the same parameter are not significantly different at 5% by DMRT.

Table 5.Yield of lowland rice in three farmers sites in luzon. 1994

SITE	TREATMENT	GRAIN YIELD (Tons/ha)
Parian, Calamba, Laguna	BIO-N	5.0a
	BIO-N + 22.5kgN/ha	3.9b
	45 kgN/ha	3.0c
Labo, Camarines Norte		
(Bicol Region)	BIO-N	1.4a
	BIO-N + 45 kgN/ha	1.4a
Los Banos, Laguna	BIO-N + 45 kgN/ha	3.3a
	90 kgN/ha	3.3a

Means followed by the same letter within a site are the same at 5% level.

Note: Yield from full rate of 90 kgN/ha for Cam. Norte and BIO-N for Laguna were harvested by the farmers prior to the schedule date of harvest.

Table 6. Average farmers yield from five different sites using BIO-N as inoculant for C-4 rice variety. Infanta, Quezon. 1997.

NUMBER OF SITES	VARIETY	TREATMENT USED	YIELD (Tons/ha)
5	C-4	T-1=Organic fertilizer +56 kgN/ha (Farmers practice)	4.8
		T-2=!/2 Farmers practice + BIO-N	6.3 (31.2)
		T-3= Inorganic fertilizer (56 kgN/ha + BIO-N)	7.00 (46.0)

Figures in ( ) are % increases over the Farmers fertilization scheme.

Table 7. Farmers data on different traditional varieties using different fertilizer inputs with BIO-N. Infanta, Quezon.1997.

FARM NUMBER	VARIETY	FERTILIZER SCHEME	YIELD (Tons/ha)
1	RC 14	BIO- N	4.7
2	Masipag	BIO-N + 28 Bags organic	5.2
3	Masipag	BIO-N + 5 Bags 45-0-0 (112.5 kg N/ha)	5.5
4	RC 14	BIO-N + 8.0 Bags Organic (40 kgN)	3.8
5	RC 20	BIO-N + 4.4 Bags 45-0-0 (99 kgN/ha)	5.3
6	RC 20	BIO-N + 5 Bags 14-14-14 + 3.3 Bags 45-0-0 (109kgN)	5.1
7	RC 14	BIO-N + 5.35 Bags Organic 45-0-0(120kgN/ha)	2.6
8	RC 14	BIO-N + 2.0 Bags Organic + 1.8 Bags 16-20(24 kgN)	10.0
9	M69	30.42 Bags Organic + 6.08 Bags 45-0-0	4.5
10	M 69	BIO-N + 6.23 Bags 45-0-0 (140 kgN/ha)	4.5

Average yield = 5.12 tons

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PROGRESS REPORT:

Project Title: The Use of Nuclear Techniques in Fertilizer-N Use Efficiency and Crop Management in Rice

Introduction:

Rice and corn are the staple food of majority of the Filipinos. It is a fact that the land area devoted to these crops has been decreasing annually due to land conversion but the population of the country continuous to increase. The current population of the Philippines is 80M mostly dependent on rice and corn and so there is an urgent need to improve crop productivity per unit area to meet the growing demand for good.

The government has been encouraging all agricultural sectors to search for fertilizer substitutes not only to reduce importation but which are also environment friendly. Some alternatives were focus in the utilization of farm wastes, composts and biofertilizers. Very recently, researches not only on rhizobia legume symbiosis, azolla, mychorriza, blue green algae , sesbania but also the living microorganism (N-fixing bacteria for rice and corn) were encouraged and financially supported in collaboration with the Department of Agriculture and Department f Science and Technology.

Through harvesting and some soil and residue mismanagement, nitrogen can get depleted and it is only through application of inorganic forms that the lost of N can be replenished. A more environment friendly practice is the use of nitrogen fixation. Biological Nitrogen Fixation (BNF) is a natural process that can recycle the unavailable nitrogen into available N and convert it into a form for plant use through the action of certain species of bacteria, actinomycetes and blue-green algae. BNF was originally recognized to exist in the legume-Rhizobium symbiosis but this process has now proven to also exist in the grass-bacteria system. The search for alternative fertilizer technologies has been started since the establishment of the National Institute of Molecular Biology and Biotechnology (BIOTECH) in 1979. It has developed inoculants for rice and corn in 1985 and even in vegetables.

Isotope and related nuclear techniques such as use of ^{15}N has played a significant role in finding crop technologies and nutrient management practices which have contributed substantially to increase rice and corn production. ^{15}N is a non-radioactive isotope of N used as a unique tracer in many studies.

Objectives:

1. To determine the efficiency of N-uptake from inorganic N and bio-N products by rice.
2. To determine the efficiency of using-low inputs technology in sustaining crop ield levels.
3. To evaluate the potential of nitrogen-fixing bacteria by rice and quantify the N-fixation using isotopic technique.

Materials and Methods:

A field experiment is being conducted at the Bureau of Soils and Water Management (BSWM) Research Station in Dalwangan, Bukidnon. The total area measures 16 m x 31m. Yield and isotope plots measure 4m x 4m and 0.8m x 0.8m respectively. There are two open top boxes installed as isotope plots per main plot for sampling at between panicle initiation and flowering and maturity stages of rice. Initial soil sampling was done at the onset of the experiment for soil characterization and fertilizer recommendation.

Experimental Design: RCBD with 3 replications

Treatments:

T1 = Control (untreated)
T2 = Full Recommended Rate
T3 = Half Recommended Rate
T4 = Full Recommended Rate + Bio N
T5 = Half Recommended Rate + Bio N
T6 = Bio N

Rate of Application:

N = 10 kg/ha
P = 7 kg/ha
K = 7 kg/ha

Fertilizer Application:

10 atom % ^{15}N was applied in broadcast inside the isotope plots based on the above treatments and fertilizer rate recommendations. Ordinary ammonium sulfate was also applied in the remaining yield plots including basal requirement of P and K except for treatment T1 (control) and T6 with Bio N only. All fertilizers were applied at planting.

Harvesting and Sampling:

Sampling will be done at between panicle initiation and flowering stage growth of rice and maturity. Samples collected will be weighed, dried at $65^{o}C$ for dry matter yield determination and ground prior to chemical and ^{15}N analyses using JASCO analyzer. Total N, P and K will be determined using the standard analytical and instrumental analyses. Agronomic and laboratory data will be analyzed statistically using the SAS method in Agriculture.

Results:

First plant sampling between panicle initiation and flowering stage will be on first week of August. Thus, agronomic and laboratory data are still in progress.

Future plans: (The Philippines)

1. Crop to be inoculated

- Corn
- Rice

2. Species of bacteria to be used:
   Genus: Azospirillum
   a. Isolates Bos 179
   b. Isolates BSs202

3. Locations/areas where BIO-N has been adopted by farmers:

   a) Laguna
   b) Rizal
   c) Cavite
   d) Mindoro
   e) Pangasinan
   f) Tarlac
   g) Ilocos Sur
   h) Cebu
   i) Tangub City

4. Philippine Nuclear Research Institute will spear head the collaboration on the Irradiation of carrier for the preparation of inoculant.

5. Collaboration with the BIOTECH, UPLB Los Banos, Laguna and farmer cooperator was started last cropping season.