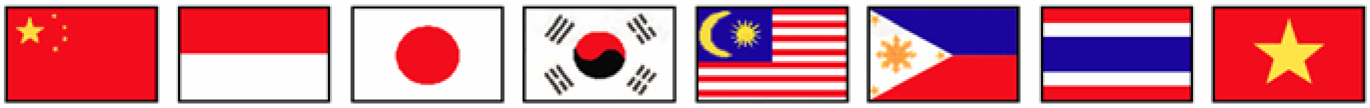

FNCA Biofertilizer Newsletter

Issue No. 6
November 2005

Message from Indonesia

Dear Colleagues,

In a country with high population like Indonesia, an increase in food crop production is the first priority for the government to fulfil the need for food. The use of plant genotypes with high productivity and responsive to fertilizer input are the normal steps undertaken to achieve this purpose. Referring to FAO Statistical database, it was reported that during the period of 21 years fertilizer consumption in South East Asia increased 3.5 times, from 10 million ton in 1976 to 45 million ton in 1997.

Beside inefficient fertilizer use by crops, excessive application of N fertilizers imparts negative impact to the environment. The remaining fertilizer N is stored in soil organic matter, some is converted to atmospheric nitrogen, and some is leached into the groundwater as nitrate pollutant.

It is well accepted that the use of bio-fertilizer could reduce chemical fertilizer dependency and produce equal grain yield as the recommended rate of chemical fertilizer.

Use of suitable bio-fertilizers to complement chemical fertilizer is to be encouraged as it will improve the natural N balance in the environment.

Issue 6 of the FNCA Bio-fertilizer Newsletter presents results from the use of bio-fertilizer in the agricultural sector in Indonesia. It is hoped that this sharing of information and the promoting of a "green biotechnology" will be beneficial to us and to those involved in the agriculture industry.

Regards,

Soertini Gandanegara

FNCA Biofertilizer Project Leader
of Indonesia

DEVELOPMENT TECHNOLOGY AND COMMERCIALIZATION OF *EMAS* (Enhancing Microbial Activity in the Soils) BIOFERTILIZER

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Introduction

Large areas of marginal soils in Indonesia have been developed for plantation. These soils have been characterized by low organic matter content, predominant low activity clays, and strongly acid in reaction. These in effect will depress the microbial activities which in turn disturb the nutrient cycle in the soils. On the other hand, under humid-tropic conditions, fertilizer loss through leaching, volatilization, and/or fixation represents an economic loss as well as a potential environmental contamination. The inefficient use of conventional fertilizers is still a part of the current agriculture practices. However, crop productivity is closely related to the ability of plant roots to extract water and nutrients efficiently out of the soils. The function of the roots is governed by an integrated set of biological processes.

A biotechnological approach is then assumed to be able to enhance the emergence of biotechnology in soil management provides a new approach in tackling efficiently many problems which remain unsolved by current conventional technology. Manipulation of soil microbes offers an efficient technique to stabilize soil aggregates, increase nutrient uptake, control soil-borne pathogen, and accelerate the decomposition of solid organic wastes, without adding new pollutants to the environment. Biofertilizers are basically microorganisms which can improve the availability of nutrient to plants. They have been believed to be an important component of sustainable agriculture, as they can reduce significantly the use of chemical fertilizers. In addition, microbial inoculant is believed to be potentially effective in inducing microbial activities in the rhizosphere. It is hypothesized that by improving rhizospheric microbial activities via biofertilizer application, nutrient solubilization can be enhanced and consequently less conventional fertilizers will be needed.

Development of *EMAS* biofertilizer technology

Research results in our laboratory (1994-1995) gave one prototype of biofertilizer product. Our previous study indicated that multi-isolate inoculums were more effective in improving soil nutrient availability and stable aggregate formation than that of the single-isolate one. Based on this evidence a biofertilizer formulation was prepared in granular form consisting of non-symbiotic N-fixing bacteria *Azospirillum lipoferum* (BCC 2369) and *Azotobacter beijerinckii* (BCC 2368), and phosphate-solubilizing *Aeromonas punctata* (BCC 2367) and *Aspergillus niger* (BCC F194). The formers have also the capability to solubilize hardly soluble phosphate, whereas the latter species can induce the formation of stable soil aggregate that intended for increasing fertilizer use efficiency on marginally suitable soils. Some of them have a capability to improve other nutrients solubilization. All of these microbes were native to Indonesian Ultisols soil. Pilot scale development of *EMAS* biofertilizer technology was constructed based on the previous results in collaboration with Project Management of the Ministry of Research and Technology, Republic of Indonesia, and state –owned plantation enterprises PTP Nusantara I, IV, VII, VIII, and XIV (1996 -1999). The goal of this project is to provide the planters by an efficient biofertilizer product capable of reducing conventional fertilizer requirements commonly applied on marginally suitable soils.

The pilot scale production technology of *EMAS* biofertilizer is an intermediate step before entering commercial scale within a series of technology development that was beginning from laboratory and green house experiments. These activities were done in 1996-1999. Development of pilot scale *EMAS* production technology was carried out on the basis of an efficient principle of bioprocess technology that means a simple technology, cheap and abundant of materials, and an effective product output.

EMAS was prepared in granular form having sizes of 2-4 mm in diameter by using a mixture of minerals and in-land peat as carrier, and packed in a 25 kg bag (Fig 1 and 2). Field experiments were conducted in plantation crops (tea, rubber, cocoa, oil palm, and sugarcane), food crops (paddy and corn), horticulture (potatoes), and spice. Application of *EMAS* has shown that it can reduce the use of conventional fertilizer up to 50%. In addition, introduction to soil provides a more stable soil aggregate and improve soil fertility status. The other benefits of *EMAS* are : (i) economically offer 10-30% saving on fertilizer cost, (ii) environmentally friendly practice, and (iii) applicable to any types of crops. Finally, multiplier effects of *EMAS* application could be generated from cost savings on storage, labour, transportation, etc. The process was patented in 1998 in Indonesia with Patent No. ID 0 000 206S.

Although the benefits offered are remarkable, the implementation of *EMAS* biofertilizer has some constraints. Being a new product type it raised a doubt about its effectiveness compared to conventional products. There are a very large number of different microorganisms in biofertilizer products. They tend to be heavily promoted and this is a great need for a standard for simple and accurate ways of measuring their effectiveness. Therefore, successful commercialization of such product needs a smart strategy to be taken. For commercial purposes, a consortium of PTP Nusantara III, V, VII, and VIII built a company called PT Bio Industri Nusantara (Bio Nusa) on 10 November 1999. The company office locates in Bandung, whereas manufactory of *EMAS* is at Purwakarta, West Java. Production technology of *EMAS* by PT Bio Industri Nusantara is under license of Biotechnology Research Institute for Estate Crops (BRIEC). This product has been legally registered for commercial production and marketing at Department of Agriculture with registration number of G 798/BSP/X/2001.

Impact on productivity

Since 1997 the biofertilizer *EMAS* has being produced semi commercially at 10 ton/day capacity. An up-scaled plant has been constructed by PT BioIndustri Nusantara (Persero) with 10,000 ton/year capacity. Unlike chemical fertilizer, *EMAS* biofertilizer will only be produced after the users order the product, since microbes of *EMAS* biofertilizer has shelf-life about 12 months.

EMAS biofertilizer has been proven to

highly potential for increasing fertilizer use efficiency of hevea rubber through the reduction of conventional fertilizer dosage on highly weathered tropical soils. This result indicated that the microbes had played an important role in improving efficient of fertilizer use. Similar phenomena were observed at other crops species, i.e. tea, cocoa, and sugarcane. In addition, at various oil palm growing environments, combination of 250 g *EMAS* biofertilizer per tree per semester and complete conventional fertilizer at a 75% reduced rate per tree can give comparably similar fresh fruit bunch (FFB) yield to full rate application of conventional fertilizers.

The justification of economic feasibility for *EMAS* biofertilizer application is based on cost reduction of fertilizer. *EMAS* biofertilizer could save national fertilizer cost at Rp. 1.5 T/year value. Results of financial analyses indicated that the development of *EMAS* manufacture with Rp. 9.625 billion investment fund could reached NPV at Rp. 14.23 billion, 72.21% IRR, 1.30 B/C, and positive cash flow cumulative in the fourth years or twenty months after production. These indicated that the manufacture of *EMAS* biofertilizer could reached a positive NPV, IRR value higher than bank loan (commercial) and B/C > 1 and therefore it is feasible to be done.

Figure 1. *EMAS* biofertilizer packed in a 25 kg bag.

Figure 2. *EMAS* biofertilizer in granular form with one year stable quality guarantee.

Concluding Remarks

Lack of intensive microbial activities in Indonesia marginal soils can be overcome by introducing beneficial microbes. *EMAS* biofertilizer that contained inoculants native from Indonesian Ultisols are proven to be cost effective, eco-friendly and renewable, and generally capable of reducing chemical fertilizers use in sustainable agriculture and plantation system. Development of *EMAS* production technology was carried out on the basis of an efficient principle of bioprocess technology. This product has been legally registered for commercial production and marketing at Department of Agriculture. Through appropriate management of *EMAS* biofertilizer in agriculture and plantation soil quality could be maintained and fertilizer use efficiency of plantation crops, food crops, and horticulture could be increased by reduction of conventional fertilizer dosages on highly weathered tropical soils.

Role of Ectomycorrhizal Fungi and Organic Fertilizer To Improve the Productivity of Tailing Soil in Gold Mining Area

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Background

Gold mining produces two types of waste; those are rocky-dump soil and tailing soil. One of serious problems in gold mining activity is the over capacity of tailing dam to store the tailing soil. In addition, restoration of tailing soil is the most difficult aspect on reclamation of mining area. Chemical characteristic such as acidity, alkalinity, salinity, and high concentration of heavy metals are common problem in gold mining. The uptake of the metals, such as lead, by the plant is seldom a problem. Association of lead and cellular or sub-cellular membranes of the plant often results in the manifestation of toxic lead effects such as reductions in the activity of phosphatase enzyme and enzyme-bound ATP-ase. To minimize negative effect of tailing, inoculation mychorrhizal fungi and addition of organic fertilizer in the tailing medium to accelerate biogeochemistry processes was applied.

The aims of this research were to study the interaction between ectomycorrhizal fungi and organic fertilizer in the tailing medium to increase the plant growth in greenhouse condition.

Material and Method

The research was conducted in Silviculture Laboratory of SEAMEO-BIOTROP and tailing soil was taken from Gold Mining Area in Pongkor-Bogor, West Java. The experiment was conducted for 8 months. Factorial experiment in Completely Randomized Design 5 x 3, with 12 replicates, was used at the greenhouse test. Two factor of this experiment consisted of type of media (five levels) and mycorrhizal fungi (three levels). The type of media consisted of tailing 100% (T1), soil 100% (T2), mixed of tailing: compost = 1:1 (v/v) (T3), mixed of tailing: compost = 3: 1 (v/v) (T4), and mixed of tailing: soil: compost = 1: 1: 1 (v/v/v) (T5). Species of ectomycorrhizae consisted of no mycorrhizal inoculation (M1), inoculated with *Scleroderma columnare* (M2), and inoculated with *Rhizopogon luteolus* (M3).

Seeds of *Shorea selanica* were germinated in sterilized soil media at green house conditions. Prior to the seed germination, seeds were sterilized using fungicide-containing Mankozeb 80% to kill pathogenic fungi. Seedling transplantation was done three weeks after seed germination into culture media according to the treatments. Mycorrhizal fungi inoculation was done one week after seedling transplantation. Inoculum suspension of one milliliter containing 10000 spores/ml was injected into media closed to the root system. The experiment was conducted at greenhouse conditions. Height and diameter growth of seedlings was measured periodically, while presence of Hartig's net and mantle structure and the seedling dry-weight were measured at the end of the experiment. Histological analysis of suspected mycorrhizal roots was done at laboratory.

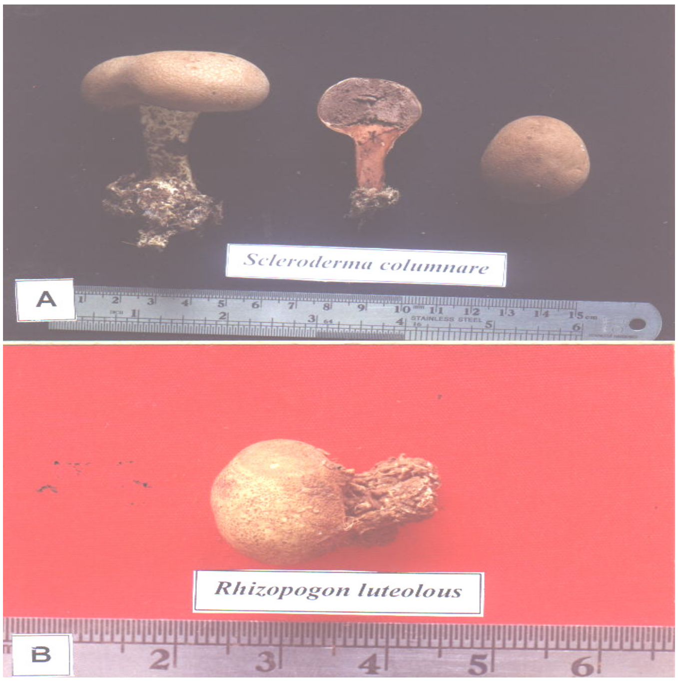

Fig 1: Fruit-body of *Scleroderma columnare* (A) and *Rhizopgon luteolus* (B)

Results and Discussion

The result of this research showed that the height growth, diameter growth, and mycorrhizal colonization of *Shorea selanica* seedlings were affected by interaction between mycorrhizal fungi and type of tailing media, but, total dry weight was only affected by single factor of mycorrhiza or type of media only.

Figure 2: Effects of ectomycorrhizal fungi and tailing media on the growth of *Shorea selanica* seedlings

The Growth of *Shorea selanica* Seedlings

The highest growth of *Shorea selanica* seedlings was obtained in the seedlings inoculated with *Rhizopogon luteolus* and added with 25 % organic fertilizer to the tailing media (T4M1). This height growth of *S. selanica* increased 272% higher than control (T1M1). On the other hand, inoculation of *Scleroderma columnare* and *Rhizopogon luteolus* with addition 25% organic fertilizer (T4M2 and T4M3) stimulated the best diameter growth of *Shorea selanica* compared to the other treatments. In this case, the diameter growth of *S. selanica* increased until 93% and 100% compared to control, respectively.

Application of mycorrizal fungi and organic fertilizer increased the total dry weight of *S. selanica.* Inoculation of *Rhizopogon luteolus* increased the dry weight of *Shorea selanica* until 8.8% higher than control or the seedlings inoculated with *Scleroderma columnare.* Mixture of tailing and organic fertilizer (3: 1; v/v) increased total dry weight of *Shorea selanica* until 196% higher than other composition of tailing media and organic fertilizer. The growth of *Shorea selanica* at different types of tailing media and mycorrhizal fungi is presented at Figure 3, 4, 5, and 6.

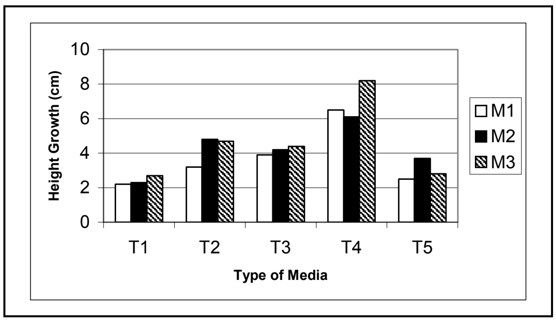

Figure 3: Effect of media on height growth of *Shorea selanica* seedlings

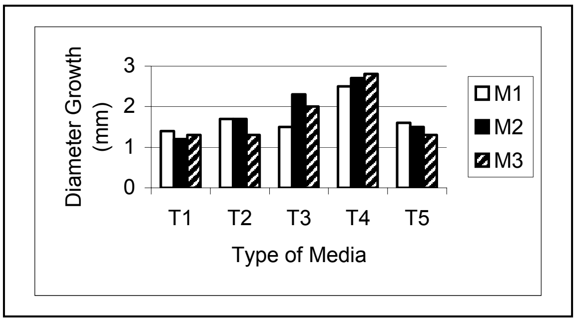

Figure 4: Effects of media on diameter growth of *Shorea selanica*

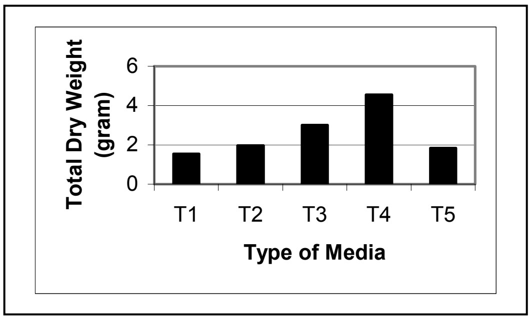

Figure 5: Effects of media on total dry weight of *Shorea selanica* seedlings

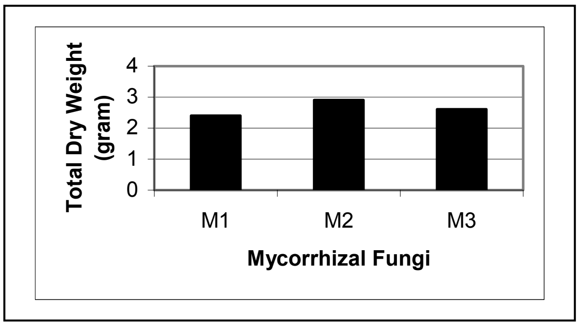

Figure 6: Effects of mycorrhizal fungi on total dry weight of *Shorea selanica* seedlings

Mycorrhizal Colonization

Mycorrhizal colonization is the key success in the use of mycorrhizal technology. Morphologically, the colonization success can be observed through the presence of hyphae enveloping the root system (Figure 7). White hyphae developed well in the seedling media. Good colonization will strengthen the compactness of culture media due to the development of hyphae and rooting system. In this case, mycorrhizal fungi release the auxin that will affect to root ramification and elongation (Brundret *et al.* 1996).

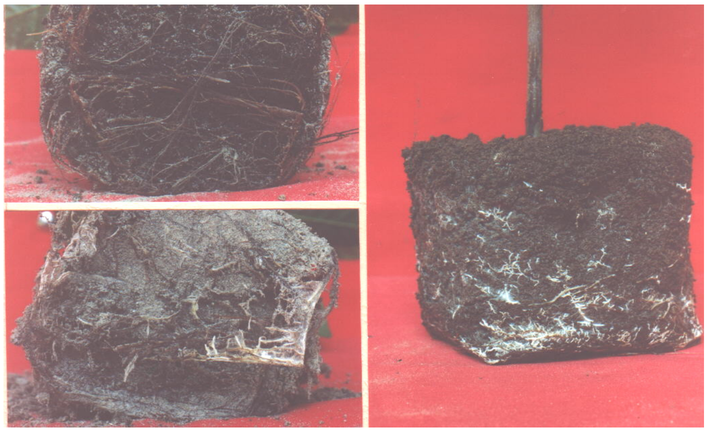

Figure 7: Non infected root (A), infected root by *S. columnare* (B), infected root by *R. luteolus* (C) in tailing media

The colonization of *Scleroderma columnare* (M2) and *Rhizopogon luteolus* (M3) can be seen in Figure 8, while their anatomical analysis can be seen in Figure 9.

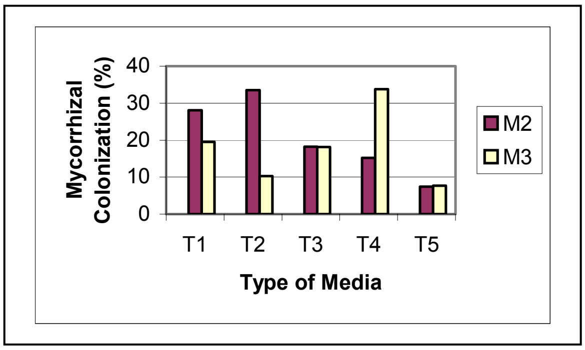

Figure 8: Colonization percentage of *Scleroderma columnare* (M2) and *Rhizopogon luteolus* (M3) in different media

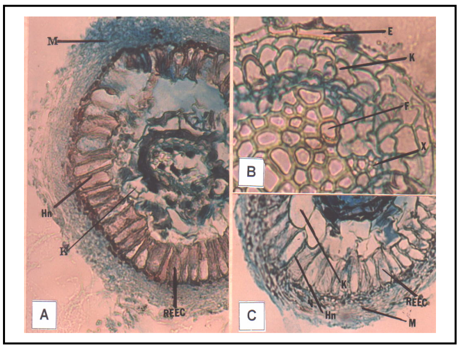

Figure 9: Root of *Shorea selanica* infected by *S. columnare* (A), non-infected root (B), and root infected by *Rhizopogon. luteolus* (C)

The average percentage of colonization by *Scleroderma columnare* and *Rhizopogon luteolus* was 28.1% and 19.5%, respectively. It can be classified as low colonization percentage. Even though their colonization percentage was low, but they were able to increase the growth of *Shorea selanica* seedlings in the culture media containing tailing as a waste of mining activity. It means that mycorrhizal fungi play important role to increase the productivity of tailing media in its association with root system of *Shorea selanica.* Good colonization of *Scleroderma columnare* was also found in pure tailing media (28.1%) and in pure soil media (35.5%). On the other hand, good colonization of *Rhizopogon luteolus* was also found in mixed-media of tailing and organic fertilizer = 3 : 1 (33.8%).

In cross-section of an ectomycorrhizal roots of *Shorea selanica* showed the presence of Mantle and Hartig's Net (Figure 9). Mantle sheets of both mycorrhizal fungi was formed in two layers, while in control was not formed. Colonization of mycorrhizal fungi in Dipterocarpaceae is very specific (Pampolina *et al*. 1994). The presence of auxin released by mycorrhizal fungi was able to develop the elongation development of cortical cells. It is not the case in *Pinus merkusii* or in Pinaceae in general.

Conclusions

Ectomycorrhizal fungi plays important role to improve the productivity of tailing soils as a waste of gold mining activity. Organic fertilizer amendment is still needed to stimulate the biogeochemistry process.

<References>

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Pampolina, N.M., R.E. de la Cruz and M.U.Garcia, 1994, Ectomycorrhizal roots and fungi of Philippine Dipterocarps. ACIAR Proc No. 62. On Mycorrhizas for Plantation Forestry in Asia.

Brief Information on Biofertilizer Studies conducted at BATAN

Azolla AS AN ALTERNATIVE NITROGEN SOURCE FOR RICE CROP PRODUCTION

Lowland rice is one of the main sources of the staple diet in the east, south, south-east Asia including Indonesia. The main constrain of this lowland is the soil fertility that is unsustainable. This is very important due to the fact that currently and in the future the lowland rice production will have to be accelerated, to meet the needs of the growing population in this region. It is observed that at lowland soils, the soil organic matter (SOM) and the soil nitrogen content are limited if not in deficiency. One effort needed to meet this limitation is to apply chemical fertilizers especially N-fertilizer, at high rates. But the risk of using N-fertilizers at high rates is its low efficiency and the potential pollution of ground water caused by nitrate.

To solve this problem is to decrease the rates of the chemical fertilizers and to replace a part of the chemical fertilizers by an alternative N source. One of the alternatives N source is biofertilizers. One of the biofertilizers which have a great potential as a nitrogen source and to build SOM is green manure. From years of study done by BATAN, *Azolla* is a suitable green manure as an alternative nitrogen source and a source of organic matter.

Azolla is a water fern plant. What has been studied by BATAN is the mini *Azolla* with a size of 3 – 4 cm. *Azolla* live in symbiosis with a Cyanobacteria which is able to fix N_2-air. The Cyanobacteria is *Anabaena Azollae*. This symbiosis makes *Azolla* to have a quite high N content. *Azolla* is indigenous in Asia, America, Europe and Africa. It has been widely used for centuries in China and Vietnam as nitrogen-source for lowland rice.

Azolla in lowland rice

Ability of *Azolla* a nitrogen-source

An international research sponsored by the International Atomic Energy Agency (IAEA) where Indonesia (Batan) participated has used ^{15}N to shown the ability of *Azolla* to fix N_2. It was found that *Azolla*, which symbiosis with *Anabaena Azollae,* could fix N_2 at a rate of 70 – 90%. The N_2-fix, which accumulated in the *Azolla*'s body, is used as altternative N-source for lowland rice.

Nutrients content of *Azolla*

Nutrients	content (%)
N	1.96 – 5.30
P	0.16 – 1.59
K	0.31 – 5.97
Ca	0.45 – 1.70
Mg	0.22 – 0.66
S	0.22 – 0.73
Si	0.16 – 3.53
Na	0.16 – 1.31
Cl	0.62 – 0.90
Al	0.04 – 0.59
Fe	0.04 – 0.59

	ppm
Mn	66 – 2944
Co	0.264
Zn	26 - 989

Several studies showed that the growth rate of *Azolla* is 0.355 – 0.390 gram per day in the laboratorium and 0.144 – 0.860 gram per day in the field. Generally in the field *Azolla* reach maximum biomass after 14 –28 days after its inoculation. Studies done by BATAN showed 3–4 weeks after inoculation at rate of 200 g fresh *Azolla* per m² the whole field at the water surface would be covered. When the whole field is covered by *Azolla* it could be expected that it could contribute 30 -45 kg N per ha or equal to 100 kg urea. It was found that *Azolla* grow better in the rainy season as long as water is available.

The benefit of *Azolla*

- Source of N which could replace urea up to 100 kg
- Feed for fish and small animals, especially chickens and ducks
- Suppress growth of weeds
- As an ornamental water plant
- Control of mosquitos
- After using of *Azolla* for four seasons or more in lowland rice it could replace P-fertilizer up to 50% from the usual rate applied.

Azolla in basin and Field trial

Research result by BATAN

A layer of *Azolla* at the water surface in lowland rice could replace 50 to 100 kg urea depending on growth rate and season.

	Production of lowland rice (ton ha^{-1})
1. Without *Azolla* layer + 50 kg urea	5.0.
2. *Azolla* layer + 50 kg urea	6.0.
3. *Azolla* layer + 150 kg urea	6.5
4. Without an *Azolla* layer + 150 kg urea	6.0.

1. Result from research in Special Province of Jogjakarta

The research in this location is still in progress. Preliminary data that can be shown from the experiment in this location are the dry weight data of grain and straw. For the time being, the highest dry weight of grain and straw – as can be seen from table – are obtained from Treatment B (100% urea).

Dry weight data of grain (kg ha^{-1})

Treatment	Replication				ρ
	I	II	III	IV	
A	4292.5	3871.25	4805	3543.75	4128.125
B	8420	6080	5642.5	6627.5	6692.5
C	7450	5673.75	6238.75	6671.25	6508.438
D	8895	7080	5601.25	4507.5	6520.938
E	4676.25	7527.5	6138.75	6558.75	6225.313
F	5596.25	7537.5	5080	6965	6294.688

2. Result from South Kalimantan

The research in this location is still being carried out until now. Preliminary data that can be shown from the experiment in this location are the dry weight data of grain.

Dry weight data of grain (kg ha^{-1})

Treatment	Replication				ρ
	I	II	III	IV	
A	3520	3440	3680	3200	3460
B	3840	4240	4480	4320	4220
C	4000	4400	4640	4720	4440
D	4620	4520	4840	4680	4665

For this time being, the highest dry weight of grain – as can be seen from table – is obtained from Treatment D (50% urea + 50% *Azolla*).

Promising Technology

Azolla is one of green manure sources, because they are (1) easily mineralized (21 – 24 days after inoculation), (2) capable of supplying enough N needed by plants (30 kg N/ha) and (3) capable of reducing the loss of N from urea and (4) could be grown by farmers going to use it. Due to the high capability of *Azolla* to fix N_2 in water, *Azolla* can be a promising source of green manure for wetland rice.

Biofertilizer for soybean on acid soil

Due to continuing land conversion of fertile soil to non agricultural purposes in Java island, the government move the areal cultivation out of Java island which dominate by low pH, low fertility and status of nutrient with high of Mn and Al content.

Soybean is sensitive to acid acidity with critical level of Al saturation around 20%. Poor soybean plant growth and no nodule formation due to ineffective indigenous *Bradyrhizobium* occurred. Soil management including liming to alleviate soil acidity is a common practice.

A program has been carried out through low input (0.5 t/ha banded on plant rows at 5 –7 cm in depth and 2.t/ha broadcasted) with soybean mutant lines and *Bradyrhizobium* inoculant develop for acid soil. Field trials were performed at upland soils in Sitiung, West Sumatra and Sembawa, South Sumatera, and tidal swamp areas at Karang Agung Ulu, South Sumatera during 1990-1996.

Inoculation with *Bradyrhizobium* improved plant performance in all locations. Evaluation on the effectiveness of symbiotic N fixation on pH ranged between 3.90 - 4.50 at low (26 –39%) and high (59 – 68%) Al saturation at Sitiung and Sembawa, Karang Agung Ulu showed that, interaction between *Bradyrhizobium* isolates – soybean genotypes significantly expressed in soil with high Al saturation. Inoculation significantly affected the grain yield in both limed and unlimed soil. It seemed that the *Bradyrhizobium* introduced were not able to establish the symbiosis in the rhizosphere in unlimed soil as good as in limed soil. Limed plots improved grain yield 136 – 161% and 135 – 246%, over control, in Sitiung and Sembawa, respectively. The multistrain inoculant showed superiority as compared with single inoculant. The grain yield reached up to 2.6 t/ha by using this multistrain inoculant.

Further screening of *Bradyrhizobium* isolates effectiveness on soil with Al saturation (50 - 68%) was carried out at Sembawa with selected soybean mutant lines. No nodulation formed in uninoculated plots has made possible to determine the N fixation by N difference method. Two local isolates (BtJ. No. 22 and BtJ. No. 37) were found to be comparable to strain TAL 102 with fixing ability of 38 – 55% and increased production of 47 – 59% over control. The production was almost double as a result of inoculation reaching 1.0 – 1.0 t/ha.

Further field trials showed that those local isolates and its mixed culture have show equal effectiveness with strain TAL 102, on nodule formation. Application of those inoculant increased plant biomass and plant N yield at R5, and grain and grain yield. Nitrogen plant yield were 114 mg N/plant and 43 kg N/ha, respectively in control plants. Nitrogen plant yield were 218 mg N/plant at stage R5 and 83 kg N/ha at harvest in inoculated plants.

Soybean mutant lines responsive to inoculation on ultisol soil were identified. Further field trials showed that especially mutant line no. 7 has some good characters. Biological nitrogen symbiotic ability of this line was not affected by high dose of N fertilizer which indicated that this mutant line is suitable for multiple cropping systems with cereal. This large seeded soybean variety has been released as Rajabasa variety in 2003.

Biofertilizer for corn

After the symbiotic N2 fixing bacteria Rhizobium, another group of soil bacteria i.e. plant growth promoting rhizobacteria (PGPR) draw attention as a biofertilizer with huge potential. Amongst them, is *Azospirillum* a gram negative bacterium, that produces plant hormones, reduces nitrate, and fixes atmospheric nitrogen (Burdman et al, 2000).

Early studies on *Azospirillum*-plant associations indicated that plant growth promotion on plant was believed to be from its N_2 fixing ability. Results from later investigations showed that the positive effects of inoculation are principally derived from morphological and physiological changes in the inoculated roots, leading to enhancement of water and mineral uptake (Fallik et al, 1994). Worldwide data of field evaluation showed that these bacteria are capable of promoting the yield of important crops in different soil and climatic regions. Around 70% occurrence of success with statistically significant yield increase in the order of 5-30% (Okon and Labandera-Gonzales, 1994). In a decade *Azospirillum* inoculant are produced in several countries for use on cereals such as rice, corn and wheat.

In Indonesia corn is an important foodcrop after rice and soybean, covering 3.000.000 ha of cultivation area, and with a production of around 9.000.000 tonne in 2004. The recommended nitrogen dose is 90 kg/ha for variety corn and 135 kg/ha for hybrid corn.

One program to obtain *Azospirillum* biofertilizer for corn was initiated in 2001 using local isolates. The effectiveness of *Azospirillum*-corn association were evaluated in green house and in field trials.

Some advantages of seed inoculation including better plant growth at early growth stage and early flowering were commonly observed. Improvement of seedlings emergence in inoculated plants were observed during drought. Three mixed inoculants increase plant growth by 27% above the control and 21% of the crop N came from the atmosphere, as determined by ^{15}N dilution method. In the field the biofertilizer could reduce fertilizer inputs, either of N chemical fertilizers or farmyard manure. Increase of production depends on soil fertility status, which ranged between 17 and 22%, and save N fertilizer close to 45 kg N/ha (100 kg urea).

Currently, at a demonstration plot at Agro Techno Park, South Sumatra a trial is conducted to evaluate selected *Azospirillum* mixed inoculant M5 on corn. Evaluation and promotion on the use of M5 inoculant on sweet corn will be carried out at farmer's field at Nanggung village, West Bogor with the cooperation of Farmer's Association.

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Sterilization of biofertilizer carrier by irradiation

Carrier material is an important factor in biofertilizer production, since it is also used as the growing media of target microorganisms during storage and coated onto seeds (Date and Roughley, 1977). Materials for carrier selected should be not toxic to the organisms, abundant and cheap. Better quality of sterile product, encouraging better growth and survival at high storage temperature, high optimum moisture content, and ease in quality control are the advantages of sterile carrier (Roughley, 1988). Sterilization by gamma irradiation is one way to get sterile carrier materials, it is the preferred method for carrier sterilization over autoclaving where irradiation source is available (Somasegaran and Hoben, 1985). Gamma irradiation process makes almost no change in physical and chemical properties of the material. While in sterilization by autoclaving, some material change their properties and produce toxic substance to bacterial strains (Somasegaran and Hoben, 1985, Senoo, in press).

In Indonesia, the use of irradiation to sterilize biofertilizer carrier was started by a private company, PT Rhizogen Indonesia, as early in 1986 for sterilizing carrier of inoculants for legumes. Irradiation was chosen due to its practical and effectiveness. The product was irradiated at the Center R&D of Isotopes and Radiation Technology, BATAN. Lower dose than the recommended dose 50 kGy i.e. at 35 kGy was chosen to eliminate *Bacillus,* a spore forming bacterium, found in dry peat. After irradiation, viability of inoculated *Bradyrhizobium* cells was found to be high, with low contaminant, after 6 month storage time.

An adhoc meeting on irradiation of carrier for biofertilizer, sponsored by MEXT and FNCA was held in Tokyo 19 - 22 June 2005, to encourage the use of ^{60}Co irradiation for sterilization of biofertilizer carrier in FNCA member countries. Irradiation sterilization is better, more economical and more practical than heat autoclave especially in case of larger production. Method of irradiation was discussed to reduce the irradiation dose was given by Takasaki Research Institute, JAERI to reduce biofertilizer cost resulting from sterilization by irradiation.

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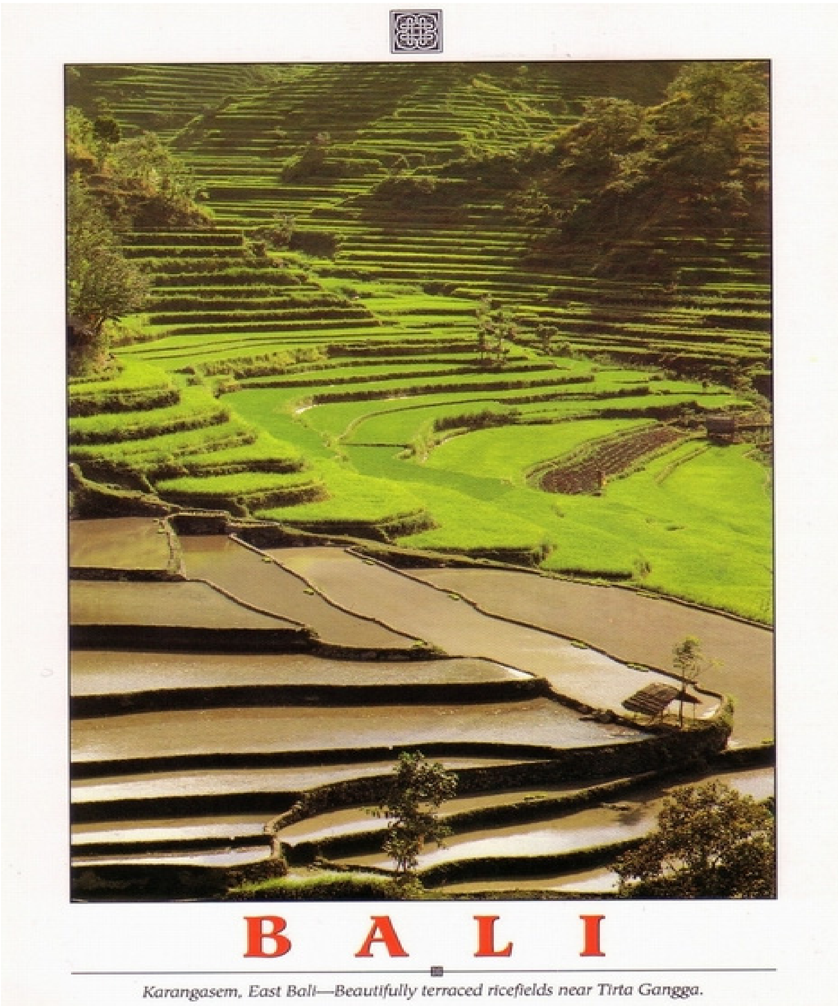

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Research Interest:

- Studies on the Use of Nuclear Techniques (32P. 14C, and 15N) in plant nutrition of food crops (e.g. photosynthetic rates, nitrogen fixation in legume-Rhizobium symbiosis and cereal-Azospirillum association)
- Participated in research on compatibility study between root and scion in some fruits plants,: rambutan, durian and mangosteen
- Screening plant tolerant to NOx application of biofertilizer on crop imprsurvement

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