Institut Pertanian Bogor * University of Wisconsin

GRADUATE EDUCATION PROJECT

Aid Project 497-0290

Report No. 18

FREIRE

R E P O R T

OF SHORT-TERM ADVISER

on

TRAINING AND INCENTIVE PROGRAM
ON Rhizobium TECHNOLOGY AND INOCULANT PRODUCTION

by

J. R. JARDIM FREIRE

June 7- July 15, 1982

Counterpart: Prof. Fred Rumawas

IPB/UW GRADUATE EDUCATION PROJECT
USAID PROJECT 497-0290

ACKNOWLEDGMENTS

This consultancy for technical assistance at the Institut Pertanian Bogor (Institute of Agriculture), Bogor, Indonésia, was possible as a result of an agreement for technical and scientific cooperation at the national and international level signed in 1977 between the University of Wisconsin (USA) and the Universidade do Rio Grande do Sul, Brasil (UFRGS). The University of Wisconsin is presently responsible for a USAID Graduate School Development Project at the Institut Pertanian Bogor under the leadership of Prof. John Murdock who was also the leader of the USAID project for graduate program in Agronomy at UFRGS, from 1966 to 1968.

I present my deep recognition and thanks for Prof. Murdock for the attentions received and for having been offered this extraordinary opportunity of cooperation with the rhizobiologists of Indonésia.

I present also my thanks to Prof. Fred Rumawas and his wife, microbiologist Agnes Rumawas, for the help and attentions while at Bogor. To the group of trainees in Rhizobium Technology Rasti, Gunawan, Siti, Imas, Elly, Zaimal, Suzy, Agustin, besides Agnes, I am also in debt for their friendly treat.

I hope that my modest contribution and efforts will be of some significance to the development of the Rhizobium program at IPB and progress of the applications of the Rhizobium/Legume technology in Indonésia.

3

CONTENTS

		Page
1.	Work Schedule	4
2.	Objectives and Introduction	6
3.	Gathering and Selection of *Rhizobium* Strains	10
4.	Inoculant Production	14
5.	Short Course in *Rhizobium* Technology	20
	Program developed	21
	Trainee information	22
	Student assignment - *Rhizobium* Program	26
6.	The Soybean Promotion Program	27
7.	Trips to Sumatra	28
8.	Australia - CSIRO/Visitor Program	29
9.	The University of Hawaii/AID NifTAL Project	32
10.	Conclusions	33
11.	Recommendations	35
12.	Annexes:	
	First Seminar	39
	Second Seminar	42
	Report on the Trainee Assignment	45
13.	Tables	50

WORK SCHEDULE

6/4/82	Left Porto Alegre to Rio. Left Rio to Miami/Los Angeles
6/5/82	Left Los Angeles to Singapore
6/7/82	Arrived Singapore 1:00 a.m. Left Singapore at 10:00 a.m. to Jakarta Arrived Jakarta at 2:00 p.m.
6/8/82	Contact with counterpart Prof. Fred Rumawas and arrangements at U.S. Embassy in Jakarta. Meeting with Dr. Robert Seeling, manager of AID Programs in Indonésia.
6/9/82	Trip by plane to Padang in the island of Sumatra (West) and from there by jeep 270 km to Sumatra (East) - Experiment Station of Sitium II Keban Percolan (Food Crops) - IPB.
6/10/82	Visit to experiments and area of Transmigration Project of 10,000 hectares to farmers settlement.
6/11/82	Return to Padang. Meeting at night with Governor of Sumatra Barat along with local personalities. Exchange of views on the Lime Project for Sumatra Barat.
6/12/82	Trip by plane to Jakarta and from the airport by another flight to Palembang at Sumatra (East).
6/13/82	Visit to Upang Delta Transmigration/Project.
6/14/82	Preparation and arrangements for short course to be initiated.
6/15-19, 21/1982	Short course - lecture, laboratory work, working group sessions.
6/22/82	Seminar to University staff and researchers from other institutions (Summary in annex).
6/23/82	Holiday.
6/24/82	Short course - lecture and laboratory work.
6/25/82	Visit to the Soil Research Center, Ministry of Agriculture, Bogor (Pusat Penilitian Tanah).
6/28/82	Seminar to University staff and administrators (Summary in annex). Together with the group of trainees meeting with Prof. Triwibono, leader of the Legume Promotion Project of the Research Institute for Food Crops.

6/29-30/82	Trip to Lampung, Sumatra (South).
7/1/82	Visit to Lembaga Biology Nasional (Dr. Susano Saono Laboratory). Working group sessions with trainees.
7/2/82	Working group sessions with trainees.
7/3/82	Left Bogor/Jakarta to Bali.
7/4/82	Left Bali to Sydney, Australia.
7/5-7/82	Visit to CSIRO at Brisbane.
7/7/82	Left Brisbane to Canberra.
7/8/82	Visit to CSIRO at Canberra. Left Canberra to Sydney.
7/9/82	Visit to Prof. Jim Vincent, Univ. of Sydney
7/10/82	Left Sydney to Hawaii.
7/12/82	Visit to NifTAL - University of Hawaii/AID Project at Maui.
7/13/82	Left Honolulu to Los Angeles.
7/15/82	Arrived Porto Alegre, Brasil.

OBJECTIVE OF THE CONSULTANCY

To train a core group of research workers in Rhizobium technology and to provide incentive for the formation of an interdisciplinary team for fostering the establishment of a small scale inoculant production unit at IPB.

INTRODUCTION

Saono in his very informative paper* reports that 350 leguminour plants have economic importance, and thirty of these are consumed as staple food or side dishes. Soybean, peanut, and mungbean have more importance. There is, however, a very wide consumption of other edible herbs, shrubs, and tree legumes. He also states that the major part of the protein consumption is of plant origin and that the first source is cereals and the second is legumes.

It is indeed very impressive how wide is the utilization of legumes by the people of Indonsia. They utilize them for food, animal feed, lumber, and firewood at the small farms and cover-crops and shade trees at the large rubber or palm oil plantations. Saono presents a wealth of information on the legumes used in Indonésia. It is clear that the use of legumes is popular and traditional by the long acquired knowledge of their properties and beneficial effects. However, it seems that there is little application of the available knowledge on the Rhizobium/legumes symbiosis for the attainment of higher N_2 fixation and higher beneficial effects.

It is our opinion, based on observations and experience in Latin America, that very seldom is the lack of availability of efficient Rhizobium strains would be the reason for the low gathering of the benefits of N_2 fixation by the farmers in the developing countries. We agree that:

*Saono, S. - Prospect for the development of legume inoculants in Indonésia. BioIndonésia No. 4:21-43, 1977. (Treub Laboratory, Lembaga Biologi Nasional, Bogor).

> Nitrogen fixation in agricultural land is rarely limited by the lack of availability of highly active nitrogen-fixing micro-organisms...a significant imbalance exists between basic studies of nitrogen fixation by these organisms and the investigations needed to eplain why so little nitrogen is fixed in nature and to devise means to overcome these deficiencies or inadequacies. The enormous progress made in recent years in understanding the biochemistry and genetics of nitrogen-fixing micro-organisms frequently is only leading to improve organisms when no improvement is needed in the foreseeable future. Conversely, essentially no attention is being given to overcoming the actual stresses that prevent the available, active nitrogen-fixing micro-organisms from doing what laboratory and greenhouse studies suggest they ought to do."*

We could argue only that the amount fixed is not "so little" because of the usually high fixation at the developed areas of high input agriculture or improved pastures as occurs in soybean areas in the United States and Brazil or in improved legume/grass pastures in Australia. However, it is true usually in most of the developing areas and subsistence agriculture where N_2 fixation is inhibited by lack or deficient application of the inoculation technology and/or correction of other nutrient deficiencies or stress limiting factors as soil acidity and toxicity by exchangeable Al and Mn. The sad aspect is that these developing regions are precisely in more need to overcome the low productivity in agriculture and obtain more food. And nitrogen is usually the more expensive and essential limiting element to increase crop productivity.

The point stated by Alexander is true because of the following aspects:

1. Good efficient Rhizobium strains for many legumes of economic or potential importance are available at several international or national institutions at least for the initiation of local programs.

---

*Alexander, M. - Research to Enhance Nitrogen Fixation: Misplaced Emphasis? (unpublished).

2. Efficient and competitive Rhizobium strains adapted to local environmental conditions can be gathered by simple, objective, applied research directed to the needs of the farmers.

3. In many of the developing counries there is a fairly large number of researchers working on Rhizobium and related fields as soil fertility science, but either they are working on non-immediate priority subjects or their finds are not being transferred to the farmers. There is a tremendous wasting of the potential of fairly well-trained scientists at least for the immediate purposes of causing an impact on farmer's productivity. Dispersed and isolated research work with lack of interdisciplinary and no integration of effort is common. In the more developed world this situation has no effect on the society: but this is not the case of the less developed world which has an urgent need to increase food production and to foster the development.

4. Small- to large-scale production of legume inoculants can be attained in simple and relatively cheap laboratory installations. However, as it occurs in many places, the inoculants available at the market, imported or locally produced, are sold at high prices; and there is no quality control services even on an informal basis. There is an example of a country, where in spite of the existence of several private inoculant factories, inoculants are imported on a large scale due to the bad quality of the local products.

5. In many areas of the developing countries soils of poor fertility are common and so the response to inoculation or to the native Rhizobia is limited by deficient supply of other nutrients (P, K, Ca, minor elements) and/or toxic levels of Al and Mn due to high acidity. The use of fertilizers and lime is scarce or nil due to lack of education of the farmers and high prices usually in result of nonexistence of governmental programs to provide incentive to local production and extension services. It is common to hear that the "local farmers cannot afford to apply lime and fertilizers" and no demonstrations are established on their farms to show the response to the application or no incentive or recommendation is given to apply these inputs in small fractions of the

land. When the farmers see good results, they will demand higher availability and credit facilities and there is incentive for local production or by large scale importation (e.g. of P) and the inputs will become cheaper. This sequence has happened in southern Brazil with lime and fertilizer use and production.

Production of inoculant for soybeans was started in 1950 first at the Department of Agriculture of the State of Rio Grande do Sul and then by private factories. However, field results of the inoculants were very poor in nodulation and higher productivity until 1965-67 when the farmers started to apply adequate levels of nutrients (mainly P) and to make adequate correction of high soil acidity and toxic factors.

6. There are examples of countries where Rhizobia strains are available. There is a fairly good amount of good research underway, and inoculant is produced; but, there is no transference of available knowledge to farmers and the inoculants are scarcely used (and mainly by the more educated, rich farmer) because of deficient linkages in research/extension and deficient extension services. Much of the responsiblity for this situation lies on the researchers and research organizations. There is not enough understanding that research is supported and performed not just to produce "papers" but also that the new finds be tested and put to use. In this way, researchers and research organizations, like an industrial factory, must feel responsible and interested that its products (information) reach the distributors of retailers (extension services) and through them the consumers (the farmers). Seminars, workshops, field days, etc. promoted by the research organization are some of the ways to attract extensionists.

Another important point is the existence of legume promotion programs by adequate governmental organizations. The research Rhizobium and the production of inoculants are of little use if there is no program for legume promotion which, in many cases, involves the need for integrated teams working on the research aspects, seed production, agriculture economics, extension, availability of inputs, credit, etc. The expansion and generalization of the use of legume inoculants in Australia and Uruguay can be explained by the existence of pasture

improvement programs. So the inoculant was promoted together with other inputs needed by the farmers. In Brazil, it was due to the existence of a governmental program to expand the soybean crop. And soybean is a very good legume of impact to promote the use of Rhizobium; response is quite clear in new lands. From the use for soybeans, farmers became aware of the advantages of the inoculation and nitrogen fixation of other legumes.

GATHERING AND SELECTION OF Rhizobium STRAINS:

Inoculants must be made with the best available Rhizobia strains from:

a. Requests from other laboratories such as MIRCEN Centers, NifTAL, USDA, other international centers, and of course, other laboratories in the country. These strains together with the local isolates would form the nucleus of a well-maintained and preserved culture collection.

b. Isolation and selection of locally adapted strains, native or introduced, in order to obtain strains of high efficiency in N fixation, with competitive ability against other introduced or indigenous strains and also able to multiply well in the inoculant carrier and survive at a long term in different types of soils. The objective is also to obtain wide spectrum strains able to establish efficient symbiosis with the largest possible number of cultivars of economic or experimental importance. First goal should be to obtain efficient strains and start to use them. Research to obtain competitiveness ability requires more time and so could be a further step. These adapted strains as for soybeans should be looked for from good sound modules from plants at fields of old introduction with inoculation or not and taking samples from different soil types and environmental conditions as a tentative to obtain diverse Rhizobia germplasm with diversified adaptation. By pressure of time and need of initiation of the program in between planting seasons, samples of each soil can be collected, disposed in disinfected pots and seeded to one or more cultivars of the desired legume. Isolations must then be made from at least one good nodule per pot. If the amount of soil is very small, the samples (1-5 g) can be inoculated in Leonard jars or pots with sterilized sand.

These collections of nodules or soil must also be accompanied by sampling each site for soil analysis as complete as possible (pH, lime requirement, available P and K, organic matter, exchangeable aluminum and manganese.

c. Preliminary screening in Leonard jars for comparison of the imported strains with the local isolates, in groups of 20-30 strains and including a control plus mineral nitrogen estimated to supply in excess the needs of the plant per pot (expected dry matter times the N content of the plant tissue). Depending on the availablity of greenhouse space and material, 3-5 replications and 1-2 cultivars must be used at this stage, and observations could consist only on number and weight of nodules and top part dry matter of the plants (2-3/pot). Auxiliary observations would consist of: 1) earliness of starting of fixation by observing the first treatments to show green foliage; and 2) position, size, and inside color of nodules. Harvest at the flowering stage or early pod formation (for soybean).

d. Second screening with the best 5-10 strains from the first test together with some imported ones as standards (for soybean we suggest SEMIA 587 and SEMIA 5019) against two or more important cultivars according to the local convenience and using five replications. Another treatment can also be included consisting of a mixture of the serologically distinct strains for a first test on the competitiveness between strains. Controls without and plus N have to be used.

Before starting this second screening and in case local research capability for serological work has not been developed the MIRCEN-Porto Alegre would be able to collaborate in this search for competitive strains. The cultures from the 5-10 best strains from the first screening should be sent to MIRCEN for production of antiserum and identification of serological groups. From this work would then come the recommendations for which strains could be used in the mixture treatment mentioned above because it should consist of serologically distinct strains. After running the screening, the nodules (air dried) from the multi-inoculum should be sent to MIRCEN for strain identification.

e. Third screening with 3-5 best native strains plus 587 and 5019, all efficient and serologically distinct strains, in pots with soil e.g. from rice field and upland. Soils should be corrected in relation to P, K, and minor elements. It is also recommended that the upland soils should be collected from problem and non-problem soils (in Java and Sumatra where there is interest for the soybean introduction) where there was no soybean before. It would be helpful to have a previous check on existence of native or naturalized R. japonicum on the soils to be used by planting a few pots of each soil without inoculation of the seeds. The treatments would consist of: 1) single strains inoculation, 2) mixture of the 3-5 strains plus 587 and 5019, 3) control without inoculation, and 4) control plus mineral nitrogen to check the potential of the legume and evaluate the contribution of the symbiotic fixation. More than one cultivar could be used and also the upland soil could be divided in limed and non-limed to check the behaviour of the crops and Rhizobia strains in the local stress conditions. It is also convenient that the right selection of the cultivar be used to gather information on the expected behaviour of the varieties to stress conditions as some varieties are more responsive in nodulation and yield to high fertility, high pH, and correction of toxicity problems. Observations would consist of plant dry matter, total N in the tissue, and nodule weight. If nodules from multi-inoculum treatment are going to be used for serological identification of the strains the nodule weight data should be taken from fresh or air-dried nodules. The serological test has to be run with fresh or air-dried nodules.

An important point with experiments in pots on soil recently limed is to avoid the high nitrification and inhibition of nodulation. At our laboratory for the experiments with soybean, we ran a first planting of soybean (or a grass crop) without inoculation in order to extract the extra nitrogen and then establish the experiment. For forage or cover crops, we take several harvests from the top plant parts. Usually the first harvest is discharged because there is no difference between strains due to high soil nitrogen.

Expression of the potential of the strains will depend on the adequacy of the other environmental conditions. It is then recommended to establish good conditions--such as, the relation of the plants to size of the pots (at least 2 kg); and soil fertility, moisture, and temperature in the greenhouse--for the best possible growth of the plants. It is important to avoid overheating the pots because higher than 35 degrees C is harmful for nodulation and N_2 fixation. It should be pointed out that it is common in tropical regions to build complete glass houses which are more adequate for temperate climates but in tropical regions necessitate establishing expensive and usually non-efficient cooling systems. In tropical areas, "screenhouses" (glass roofs and screen walls) would be more adequate.

Observations: 1) dry matter and total nitrogen of the plants--2) nodule weight. Auxiliary observations as for the first screening. Harvest at the flowering stage or early pod filling.

f. Fourth screening - field experiments with the same treatments as the pot experiment run parallel or afterwards at the same sites where the soil samples were taken. For these field experiments, a peat inoculant of each strain and mixture of strains should be made previously for the seed inoculation at the site of the experiment. Rate of the inoculum should be in excess (5-10 times the usual rate) to avoid failures due to unavoidable stress conditions. Correction of nutrient deficiencies except nitrogen (macro and minor elements) and Al and Mn toxicities are essential. Where necessary, irrigation would avoid failures in germination and nodulation, deficient plant growth and wasting of time and money.

At this experiment, as also mentioned for the pot experiment, cooperation from MIRCEN could be obtained for serological identification of the nodules. Twenty to forty nodules per pot or plot would be sufficient for the test of competitiveness between strains. These nodules should be air-dried and mailed in paper bags.

Observations: 1) nodulation evaluation 30 days after germination and at flowering stage (for soybean) from 10-20 plants taken at random from lines especially established for the sampling. Data

collected-nodule weight, beans yield, and N content. In soybeans, N content of the beans is an important observation to differentiate between strains.

There is a point we consider of high importance. In the developing countries, almost all the research is at the hands of governmental or university organizations. Concerning Rhizobium, the research directed toward the identification of efficient, compeletive, and locally adapted strains will have little value unless the inoculants produced in the country or imported contain these strains. Some kind of mechanism must be devised to regulate the matter. In Brazil, the federal law on legume inoculants requires that they must contain the strains proved to be effective by the official laboratories. The private industries are supplied with strains from these laboratories.

INOCULANT PRODUCTION

Legume inoculants have been prepared and used since 1896, but generalization of their use occurred almost only in the developed countries--first in Europe, then in North America, and afterwards, by the early fifties, in Australia. In Brazil, inoculant production by governmental agencies started in 1950, but large-scale private production developed in the country only after 1956.

Inoculants are already produced in Indonésia at two places. At the University of Gadja Mada, Jogyakarta (Central Java), the inoculant "Legin" is produced for soybeans and sold at the price of US$6.00 mainly to the Soybean Promotion Project. There was no precise information on the quality of this product, either on the amount produced or the amount used. At the Research Institute for Plant Industrial Crops, Medan, North Sumatra, an inoculant is being produced and experimented for cover crops.

There is no point in reproducing here the literature on the production of legume inoculants. However, we will make some observations on some more interesting aspects and recent developments.

1. Preparation of the broth culture

The preparation of Rhizobium culture does not require sophisticated or expensive equipment. Aerated flasks or even flask shakers are adequate along with other accessory items such as an autoclave, etc. However, it is important that aseptic conditions be maintained to avoid contaminated broths. Small clean rooms free of dust are recommended to avoid wasting of time by contaminations. A Lamminar flow hood is expensive, but a small aseptic chamber can be built.

On the concentration of the broth, the usual recommmendation is to mix a rich culture (500 million to 1 billion cells/ml) with the carrier. However, very recently some researchers (Corby, Roughley, Halliday) found out that if well-sterilized peat* is used, the rich broth can be diluted several times without harm to attainment of high concentration of cells in the peat. Halliday (personal communication) reported that the dilution can be as high as ten thousand times. This finding is highly significant: it is not necessary to have a large volume of broth to produce large quantities of inoculant.

For laboratory small-scale production there is no need to grow the bacteria in liquid media. Flat flasks (250-500 ml) can be used with agar media and the growth suspended in liquid media is mixed with the peat in a tray or injected in the bags. The growth on solid media has the advantage of easy checking of contamination and also of avoiding the use of fermentors or shakers. It will depend on the amount of growth obtained, the dilution that can be made, and the amount of final product. With a 250 ml flask, it can easily make 1-2 kg of inoculant.

Concerning the culture media, it can be found in the literature that most Rhizobia utilize mono and disacharides easily as a source of carbon, but the slow growers prefer pentoses (arabinose). For R. japonicum it was common to use sucrose which is much cheaper than arabinose or mannitol. However, recent developments have pointed out

---

*Sterilized already packaged by autoclave or gama irradiation.

that glycerol is a better carbon source for this Rhizobia than mannitol, glucose, galactose, and lactose; sucrose being hardly utilized. BALATTI* recommends the following formula for R. japonicum:

Water	1000 ml	(900 ml if yeast water is used)
Yeast extract	4.0 g	(or yeast water 100 ml)
PO_4HK_2	0.5 g	
SO_4Mg7H_2O	0.2 g	
NaCl	0.1 g	
SO_4Mn 10%	2 drops	
FeCl 1%	2 drops	
KNO_3	0.8 g	
$PO_4H(NH_4)_2$	0.3 g	
Glycerol	10.0 g	

2. Preparation of the carrier

In spite of the large amount of research done on alternative carriers for the Rhizobia inoculum, peat is still considered the best one. In some cases, research has been done without looking for peat around the laboratory place. Date* has described the essential characteristics of a good peat. However, there is no way to tell in advance if one peat is good or not, Rhizobia survival capacity has to be tested.

It is our experience that for small to medium scale production (up to five tons) it is not necessary to dry the peat in ovens or drum dryers. In tropical or subtropical climates, sun drying or a simple small hut constructed with zinc tiles painted black will do the job. It is important not to heat the peat beyond 100 degrees C' when an oven is used.

Autoclave sterilization is efficient either in bulk or disposed in autoclavable (polypropilene) bags.

After drying, the peat must be mixed with fine dolomite lime for neutralization to pH 6.5-7.0 and then milled.

*Balatti, A. Production de inoculants. (unpublished)

3. Mixing peat with the broth

If the peat is sterilized in bulk it has to be dispersed to ensure uniform coating of the particles with Rhizobium. The dispersion may be done in the bags either before or after mixing with the broth. If, however, a rich broth is used severe contaminations are not common. If the peat is previously dispersed in bags and then sterilized, the broth has to be injected, concentrated, or diluted as mentioned before.

*Date, R. A. Legume inoculant production. Porc. Indian Natl. Sci. Acd. Vol. 40, B, No. 6, 1976.

4. Inoculant quality control

Establishment of the facilities and systematic quality control of the available inoculants is essential and the equipment and installations can be fairly cheap. Siratro (Macroptilium atropurpureum) plants in large tubes can be used as a guinea pig legume for soybean inoculant. The disposable pouch method using soybean seeds is also accurate. Of course with sterile peat inoculants and low contamination the simpler and quicker petri dish viable counting can be used.

It is not necessary to wait for a governmental law to start an informal quality control. It must be understood that the extensionists and researchers in other fields are in many cases promoting or using inoculants of doubtful quality in respect to adequate number of cells or adequate strains. We think that the Rhizobium researchers and of course the organization that harbors Rhizobium program should feel responsible to test the quality of the inoculants in the market as a way to defend the credibility of their own work and product. It is highly convenient that the information gathered be passed to other organizations such as extension services, research stations, farmers' cooperatives, etc.

Still concerning the quality control, it is recommended that the inoculant be tested at different periods up to the expiring date and also from samples taken at farmers level after storage and transportation. Another point is that where no laboratory facilities are available, an easy way to gather some indication of inoculant quality is to run field tests with levels of application from one to several times the recommended rate.

5. Inoculum rate

The establishment of a field research program on the locally adequate inoculation rate is recommended because the availability of good strains and good inoculants are not sufficient to get good field results even where there is no nutritional deficiency or toxic factor. Inoculant standards are only an indication on the theoric optimum number of cells per gram of product or to be applied per seed. It is convenient

to run field tests on different environmental conditions and soil types to get information on the best inoculation rate according to the situation. The first planting of soybean in a field needs a much higher number of Rhizobia cells per seed than the usual recommended rate to form a good nodulation. For this situation, the usual recommendation in southern Brazil is 2-4 times the normal amount of inoculant. In central Brazil where new areas are usually "opened" by clearing of the native brush-sward cover and planting upland rice for 1-3 years and then seed soybean, the extension services are recommending that the inoculant be mixed with the rice seeds. When soybean is seeded it will already find an established population of the Rhizobia. Farmers are obtaining good results from this practice.

Another reason for higher inoculum rate at first planting is that very commonly in the tropics soybean is seeded in hot-dry soils. It is then convenient to evaluate the effects of these and other stress factors on the survival of Rhizobia in the soil and on nodulation.

6. Inoculant cost

We consider that the price of inoculants must be the cheapest possible in order to insure that the farmers will be able to use them on a large scale. However, they should not be supplied free because of the discredit involved in a "gift." Commercial inoculants for soybean currently cost around US$1.00 in USA. In Brazil, privately made inoculant for soybean costs around US$0.20. They can be even cheaper if prepared at nonprofit organizations.

7. Inoculant transport and storage

Very commonly, poor conditions of transport and storage are the main reason for the low number of Rhizobia cells in the inoculants. There is little understanding that legume inoculants carry live bacteria cells. Storage and transport under refrigeration is essential in warm climates. For short periods, insulated styrofoam boxes can be used to avoid hot temperatures during transportation.

8. Equipment and materials

The sophistication of the equipment and installations will depend, of course, on the funds available. Below we list some of the more important items:

- Laminar flow hood or culture room
- Autoclaves for laboratory one with a square door for the greenhouse
- Binocular microscope
- Refrigerator
- Freezer
- Incubator (28-30 degrees C)
- Balances
- Oven for glassware sterilization
- Reciprocal shaker
- Water stills for laboratory and greenhouse
- Plant drying oven
- Package sealer
- Hammer mill
- Sieves (100-200 mesh for peat)
- Leonard jars (easily made from wine or beer bottles)
- Kjedahl aparatus
- Water baths

SHORT COURSE ON *Rhizobium* TECHNOLOGY

The short course on *Rhizobium* technology was developed in ten days of work with the nine trainees. Each day of the course consisted of 1-2 hours of lecture and 2-3 hours of laboratory work or 2 hours of working group sessions, from 8:30 a.m. to 1:30-2:00 p.m. For these group sessions the trainees were given a special assignment for the development of a *Rhizobium* microbiology and ecology, the effect of biotic and abiotic limiting factors on N_2 fixation, the role of the legume as a partner of

the symbiosis and inoculant production technology necessary for the establishment of a program for inoculant production. The special student assignment on which the trainees worked as a group had the objective of developing in each one the concern about the problems involved in transferring of the available knowledge, utilization and gathering of the benefits of the Rhizobium/Legume symbiosis by the farmer. A copy of the trainee's report is annexed.

Program Developed

Tuesday, June 15	Acquaintance with the students and their background, field of work and interests. Lecture: Objective of the course. Legumes - importance and role in the symbioses. The microsymbiont Rhizobium, morphology, physiology, and biochemistry. Working group session: introduction for the student assignment for the establishment of a "Rhizobium Promotion Project."
Wednesday, June 16	Lecture: nodule formation, morphology, histology. Lab work: nodule collection. Rhizobium strain isolation.
Thursday, June 17	Lecture: Specifically Rhizobium/legumes. Principles of strain selection. Lab work - Rhizobium characterization. Greenhouse - Preparation of Leonard jars for strain selection
Friday, June 18	Lecture: Benefits of the symbiosis Rhizobium/legumes. Greenhouse: Strain selection - seeding and inoculation. Preparation of an experiment on need of inoculation and evaluation of limiting factors - liming of a soil brought from Sumatra Sitium II. Working group session
Saturday, June 19	Lecture: Limiting factors on the symbiosis Working group session: discussion on the trainees assignment.

Monday, June 21	Lecture - Inoculant production and control. Techniques of inoculation, evaluation of inoculation, and N_2 fixation. Lab work: Preparation of inoculants.
Tuesday, June 22	Lab work: Preparation of material for bacteria counting. Preparation of inoculants.
Thursday, June 24	Lecture: Establishment of the need for inoculation. Mechanism of the N_2 fixation. Lab work: *Rhizobia* enumeration in inoculants. Strain isolation - observation of Petri dishes and *Rhizobia* characterization. Greenhouse: Seeding of the experiment on the need inoculation and evaluation of limiting factors.
Thursday, July 1	Working group session: Presentation by each one of the trainees on their work, experiences and plans on *Rhizobium* technology or related fields.
Friday, July 2	Working group session: Final discussion with trainees on the paper presented by the group on "*Rhizobium* Promotion Project."

Trainee Information

1. NAME: Agnes Rumawas

   ADDRESS: Jln Tanjung LA - Kampus IPB
   Darmaga, P.O. Box 213
   Bogor, INDONESIA
   c/o Dr. Fred Rumawas
   Dept. of Agronomy, IPB
   Bogor - INDONESIA

   EDUCATIONAL BACKGROUND:
   Dra. - Biology I.T.B.
   MSc. - Microbiology, Univ. of Kentucky-USA

   FIELD OF WORK: Microbiology

   PREVIOUS TRAINING IN *Rhizobium*: None

   IN WHAT AREA IS THE KNOWLEDGE ACQUIRED IN THIS COURSE GOING TO BE APPLIED? *Rhizobium* production for rain-fed agriculture project.

   PUBLICATIONS IN *Rhizobium* AND OTHER AREAS: None

2. NAME: Agustin Wydia Gunawan
ADDRESS: Departemen Botani, I.P.B.
EDUCATIONAL BACKGROUND: Phytopathology
FIELD OF WORK: Mycology
PREVIOUS TRAINING IN Rhizobium: NifTAL, Hawaii, 1976
IN WHAT AREA IS THE KNOWLEDGE ACQUIRED IN THIS COURSE GOING TO BE APPLIED? Inoculation production, particularly in seed pelleting
PUBLICATIONS IN Rhizobium AND OTHER AREAS: None

3. NAME: Zainal Mahmud
ADDRESS: Jl. Cimonggu 1
Bogor, INDONESIA
EDUCATIONAL BACKGROUND:
Insinyur (Ir.) Agronomy; Andalas University, Padang, INDONESIA
Magister Sains (MS.) Agronomy; Bogor Agricultural University, Bogor, INDONESIA
FIELD OF WORK:
1. Research worker for Industrial Crops (CRIIC, Bogor)
2. Rhizobium inoculant
PREVIOUS TRAINING IN Rhizobium: Biological Nitrogen Fixation and its Ecological Bases. (January 18-29, 1982, Caracas).
IN WHAT AREA IS THE KNOWLEDGE ACQUIRED IN THIS COURSE GOING TO BE APPLIED?
- Contributions to biological nitrogen fixation for other plants (Mixed cropping).
- Rhizobium sp inoculants production.
PUBLICATIONS IN Rhizobium AND OTHER AREAS: --

4. NAME: Sri Erita Aprillant
ADDRESS: Jl. Sukamulya II/20^{A} - Bogor
EDUCATIONAL BACKGROUND: Agronomy
FIELD OF WORK:
1. Information staff at Central for Soil Research
2. Assistant research staff of soil management at Central for soil research.

4. Sri Erita Aprillant (Continued)

PREVIOUS TRAINING IN Rhizobium: At IPB

IN WHAT AREA IS THE KNOWLEDGE ACQUIRED IN THIS COURSE GOING TO BE APPLIED? Will be an assistant of microbiology lecturer at IPB.

PUBLICATIONS IN Rhizobium AND OTHER AREAS:

Resistance test of some hybrid corn variety to bulai Disease (Sclerospora maydis (RAC) BUTL) and its effect on production.

5. NAME: Tedja Imas A. Husein

ADDRESS: Subdiv. Microbiologi, Dept. of Botani - IPB

EDUCATIONAL BACKGROUND: Univ. Degree-IPB-1967

FIELD OF WORK: Lecturer in Microbiology

PREVIOUS TRAINING IN Rhizobium: None

IN WHAT AREA IS THE KNOWLEDGE ACQUIRED IN THIS COURSE GOING TO BE APPLIED?

1. Inoculant production
2. Culture collection

PUBLICATIONS IN Rhizobium AND OTHER AREAS:

1. Agronomical aspects of farmer's citrus plantations in Kabupaten Batanghari, Jambi, Sumatera, 1979.
2. The effect of NPK fertilizers on seed development of groundnuts in Batanghari, Jambi, Sumatera, 1980.

6. NAME: Elly Darliah

ADDRESS: Centre of Soil Research
Dr. H. Juanda 98

EDUCATIONAL BACKGROUND: S.A.K.M.A. (High School of Chemiesky)

FIELD OF WORK: Microbiology

PREVIOUS TRAINING IN Rhizobium: --

IN WHAT AREA IS THE KNOWLEDGE ACQUIRED IN THIS COURSE GOING TO BE APPLIED? Soil Microbiology

PUBLICATIONS IN Rhizobium AND OTHER AREAS:--

7. NAME: Gunawan Djajakirana

ADDRESS: Department of Soil Sciences
Bogor Agricultural University
Bogor, Indonesia

EDUCATIONAL BACKGROUND: Graduate from Bogor Agriculture University (IPB) in Soil Fertility

FIELD OF WORK: 1. Soil Biology
2. Soil Fertility

PREVIOUS TRAINING IN Rhizobium: --

IN WHAT AREA IS THE KNOWLEDGE ACQUIRED IN THIS COURSE GOING TO BE APPLIED?
1. Rhizobium sp production
2. Nitrogen fixation in legumes
3. Rhizobium research (inoculation)

PUBLICATIONS IN Rhizobium AND OTHER AREAS:
1. The influence of compost on the availability of Phosphorous in Red Yellow Podzolic Soils
2. Response test of tropical Legumes to the inoculation with the available strain of Rhizobium.

8. NAME: Siti Komariah

ADDRESS: Centre for Soil Research
(I.R.H. Juanda: 98)

EDUCATIONAL BACKGROUND: Academy of Analytical Chemistry

FIELD OF WORK: Assistant Microbiology Soil Research Staff Centre for Soil Research.

PREVIOUS TRAINING IN Rhizobium: At IPB

IN WHAT AREA IS THE KNOWLEDGE ACQUIRED IN THIS COURSE GOING TO BE APPLIED? Microbiology at PPT to be assistant microbiology in September

PUBLICATIONS IN Rhizobium AND OTHER AREAS: --

9. NAME: Rasti Saraswati

ADDRESS: Central Research Institut for Food Crops
Jl. Cimanggu Kecil 2 Bogor.

EDUCATIONAL BACKGROUND: Biology Graduate of Padjajaran University, 1980.

FIELD OF WORK: Soil Microbiology/Rhizobium

PREVIOUS TRAINING IN Rhizobium:

1. Microbiology Department, National Biological Institute, Indonésia Science Institute (LBN-LIPI) - Indonésia
2. Microbiology Department, Bogor Agriculture Institute, (IPB) - Indonésia

IN WHAT AREA IS THE KNOWLEDGE ACQUIRED IN THIS COURSE GOING TO BE APPLIED? The knowledge willbe applied in soybean area

PUBLICATIONS IN Rhizobium AND OTHER AREAS: --

Student Assignment

Project Planning on RHIZOBIUM Promotion Program

Objective: Attainment of higher benefits from the N_2 fixation by the symbiosis Rhizobium/legumes, economy of fertilizer nitrogen and higher yields of agriculture products.

Purpose: Enhancement of research capability, diffusion of information, inputs availability.

Means: Assessment of the situation.

1. Important legumes and of potential - grain, forage, green manure, reforestation for food and economy of the country.
   Other crops of importance - in general and by the farmer.

2. Research - institutions, personnel, financing, facilities (laboratory, greenhouse, field). Bibliography. Work underway - priorities.
3. Limiting factors - soil, credit, seeds, inputs (lime, fertilizers, etc.).
4. Legumes promotion programs.
5. Inoculants - availability, production, quality control.
6. Agrarian structure - farmers, education, size of property, etc.
7. Extension - linkages with research.

Project Planning:

1. Research.
2. Inoculant production and quality control.
3. Dissemination of information - extension.
4. Inputs availability to the farmer.

Implementation: Conclusions and recommendations.

THE SOYBEAN PROMOTION PROGRAM

Increased soybean production is one of the objectives of the Mass Guidance Intensification Project (BIMAS) under way by integrated governmental and university organizations. The area of production is rapidly expanding. It is estimated that at the present 780.000 hectares (ha) are being grown, but only 500.000 ha are being fertilized. One hundred thousand ha are being opened for soybean cultivation, but the potential is around six million ha mostly in red yellow podsolic soils. Considering all Indonésia, the potential production area is around twelve million ha.

Because of the high consumption of soybean by the people of Indonesia, there is an unsatisfied demand, which, in 1981, was around 300.000 tons. The goal of the BIMAS project is to have production of 2.3 million tons of soybean in a period of five years.

Therefore, there is an urgent need for an increased availability of legume inoculants in the country containing locally selected Rhizobium strains. Current inoculum production cannot sustain the planned expansion of the soybean crop.

TRIPS TO SUMATRA

In the visit to the Sitjum I and II of the Transmigration Project in Sumatra, we had the opportunity to see how important is the need for liming to correct acidity stress factors and N_2 fixation in soybeans. In the absence of lime, soybean nodulation and yield is very poor as was seen at the experiments ("crazy plots") at the Sitium II Experiment Station. It is clear that the attainment of high N_2 fixation, good yields, favorable economic returns to the farmers, and expansion of the soybean crop will be dependent on correction of soil acidity and fertility. Fortunately, it seems that there is a strong move among researchers and authorities to establish a liming program. At a meeting with the Governor of Sumatra Barat and other local leaders that was held at the Governor's palace in Pandang, we were highly impressed by the clear understanding of the situation and the willingness to do something.

In the trip to Lampung (Benchmark Project) at West Jawa, which is 180 km north of Nakau at the coast, the problem of soil acidity and poor fertility was also clear. An experiment with soybean and lime application at a "low input levels" (up to 1 ton/ha) the plants were showing clear symptoms of Mn toxicity and had no nodules. It is also worth mentioning that on this trip around the island of Java, we crossed large extensions of land with infertile acid soil and a very low population density. This contrasts with the crowded situation that

exists for areas of high fertility soils that are usually cropped with rice in other areas of the island. Those poor areas would be considerably more productive if the adequate inputs were made available to the farmers.

Another kind of problem was seen on the trip to the Upang delta tidal areas where farms are also being established. Here the high soil clay content and iron sulphate (cat clay) will present a severe stress condition for Rhizobia infection, nodulation, and survival in the soil.

CSIRO - Division of Tropical Crops and Pastures - Cunningham Laboratory - Brisbane

A formal program of interviews was followed according to the copy annexed. An important objective of the visit to Brisbane was to contact and gather information on the work on Rhizobium being led by Dr. Richard Date. This work has focussed on tropical and sub-tropical forage legumes which also include some legume cover-crops common in Indonésia. Dick Date has trained many students from other countries both in practical training and graduate training in conjunction with the University of Queensland. He is definitely an excellent alternative for medium- to long-term training. He also has a large culture collection of Rhizobium strains mainly for tropical legumes which can be supplied to other researchers.

Another objective was to visit the World Data Center-MIRCEN, located at the Department of Microbiology of the University of Queensland in Brisbane. We contacted Dr. V.D.O. Skerman, MIRCEN Director. This MIRCEN has offered short courses on Microbial Culture Collection and probably is going to offer another one in 1983. It would be advisable to at least have the microbiologist in charge of the inoculant production at IPB participate in this short course. It is important to point out that participation in training programs and cooperation with Australia could be supported by the Australian Organization for Cooperation with Asian Countries (OECD).

CSIRO - Division of Plant Industry - Canberra

In Canberra, I had the opportunity to interview; Dr. Alan H. Gibson, a well-known scientist who specializes on limiting factors of the *Rhizobium* symbiosis; F. Bergensen, a specialist on the N_2 fixation mechanism; and, W.F. Dudman, a specialist on *Rhizobium* identification by serological tests. Training and/or contact with these scientists would be of significance to the research in Indonesia.

In Sydney I met Dr. J. M. Vincent, one of the pioneers and leader of the *Rhizobium* science in Australia who is at present officially retired, but still active on *Rhizobium* work.

CSIRO/Division of Tropical Crops & Pastures/The Cunningham Laboratory
A Division of the Institute of Biological Resources
306 Carmody Road, St. Lucia, Qld. 4067 Australia
Telephone (07) 377-0209 Telex 42159

VISITOR PROGRAM

Dr. JOSE JERMANO STAMMEL
Dr. JOAO RUY JARDIM FREIRE

Faculty of Agronomy, Federal University of Rio Grande do Sul, Porto Alegre, R.S. Brazil.

TUESDAY, JULY 6, 1982

During the morning Dr. Stammel and Dr. Freire will join discussions with Mr. Ritchie and Mr. Abba who are also visiting Cunningham Lab.--For details see attached program.

pm program for Dr. Stammel

1.30 pm — Dr. F. W. Smith (Leader, Plant Nutrition)
- comparative nutrition of pasture species; basic nutrition of summer cropping species; diagnosis of nutrient disorders; availability of soil phosphate.

2.00 — Dr. B. A. Keating (Plant Nutritionist)
- nutrition and growth of legumes on clay soils; effect of pH on legume growth and nutrition.

3.15 — Dr. P. C. Kerridge (Plant Nutritionist)
- maintenance fertilization of grass-legume pastures; studies with rock phosphate.

pm program for Dr. Freire

1.30 pm — Dr. R. A. Date (Leader, Legume Bacteriology)
- ecology of *Rhizobium* for tropical forage legumes; factors related to nodule formation and function.

Dr. H.V.A. Bushby (Legume Bacteriologist)
- ecology of *Rhizobium* for tropical grain legumes; factors related to nodule formation and function.

3.30 — Dr. W. Jehne (Division of Soils) - Soil microbiologist.

T. W. Elich
TECHNICAL INFORMATION OFFICER.

July 5, 1982.

THE UNIVERSITY OF HAWAII/AID NifTAL PROJECT

On the 12th of July we paid a short visit to the NifTAL Project under the guidance of its Director, Dr. Jake Halliday. It is an excellent training and research center provided with all necessary infrastructure and personnel.

NifTAL has trained several researchers from Indonésia as listed below:

6-week training course

AGUSTIN GUNAWAN
Dept. of Botani, I.P.B., Bogor

TINI PRIHATINI
Soils Research Institute, Bogor

ISMAIL BRAHIM POER BOY
Sukamandi Research Institute for Food Crops
Sukamandi [a subdivision of SFRI (formerly CRIA-LP_3)].

Intern Trainees

OMAR O. HIDAYAT
Sukamandi Research Institute for Food Crops, Sukamandi

MURSIDI SEDIYARSO
Soils Research Institute, Bogor

From November 1 to December 15, NifTAL is going to offer a training course in conjunction with the Bangkok MIRCEN. It is a good opportunity to send one or more researchers for more normal training on Rhizobium technology.

NifTAL coordinates an International Network of Legume Inoculation Trials (INLIT) with a large number of collaborators in Africa, Asia, and Central and South America. According to the information collected at NifTAL, the following institutions and persons from Indonesia are collaborators in the INLIT trials:

- Institute actually conducting trials
  Central Food Research Institute formerly CRIA-LPs),
  Bogor

Director - Dr. RUSLI HAKIM

Legume Coordinator - Dr. SADIM SOMAATMADJA

The INLIT trials are normally under his jurisdiction man actually coordinating trials at various sites in the country - OMAR HIDAYAT.

- Institute that has expressed interest from beginning but has as yet put out trials

  Center for Soils Research (formerly SRI), Bogor

  Director - Dr. D. MULJADI

  Dept. Director - Dr. M. SUDJADI

  He is the person actively interested in soil microbiology.

The above information concerning trainees and INLIT trials were gathered at NifTAL. Unfortunately, it was not available before for a more accurate assessment of the in-country situation.

CONCLUSIONS

From the information and observations gathered at Bogor and the trips to the island of Sumatra, we have reached the following conclusions:

1. In spite of the old and traditional use of legumes by the Indonesian farmers, there is little use of the Rhizobium/legumes symbiosis as a way to increase legumes productivity either by the use of inoculation techniques and/or by correction of limiting factors for the attainment of higher N_2 fixation. There is, however, an increasing concern among researchers and agriculture organizations on this aspect.

2. There is a fairly large number of Rhizobium researchers in Indonesia and many of them with adequate training. Many scientific papers have been published or presented at meetings. There is, however, significant waste of trained manpower. Several researchers with specialized training on Rhizobium are working in other fields, have taken administrative jobs, or have only teaching responsibilities.

3. The basic infrastructure for Rhizobium research already exists at IPB. Considering the Bogor area, at least the Soil Research Center and the Lembaga Biology Nasional have good laboratories and personnel.

4. On the potential use of inoculation practices, it is clear that soybeans are a high priority followed by peanuts and mungbeans. For cover crops (herbs, shrubs, trees) at industrial plantations, there is also a large potential. However, as most of the industrial plantations are established on infertile acid soils, the beneficial effects from N_2 fixation will be limited by the local stress conditions.

5. The existence of governmental programs for promotion of the so-called secondary (palawija) crops is of high significance for the success of Rhizobium N_2 fixation program. Also highly significant is the promotion of soybeans in the transmigration areas. However, the field results from the use of Rhizobium inoculation will depend on the correction of the other soil limiting factors to attain high productivity on the low fertility, acid soils with Al and Mn toxicities. At the farms where soybean has been grown for a long time, an established R. japonicum population will probably limit the effect of inoculation of introduced strains.

6. Population in Indonesia is concentrated mainly in Java, in areas with fertile soils. However, there are large areas even on this island where population is sparse due to poor soil fertility. This situation of course happens in other regions of the world and most commonly the utilization of these areas is poor because the farmers have no knowledge and/or means to improve the soil fertility, to increase productivity, and to grow more profitable crops. This was the case of the upland plains area of sourthern Brazil, when it was dominated twenty years ago by poor native pastures. The "cerrado" (savanna) soils of central Brazil were also considered "useless" until technology, inputs, credit, etc. became available for the establishment of a high productivity agriculture. Soybean production is now expanding rapidly in Central Brazil and, as happened in the south after the soybeans came in, the improved soil is able to be cropped with other responsive crops or improved pastures.

7. The "bottlenecks" for a widespread attainment of higher benefits from the Rhizobium/legumes symbiosis in Indonesia seems to be: a) a large part of the research underway lacks objectives and goals for immediate application; b) low availability, high prices and lack of quality control of legume inoculants; c) extension services and legume promotion program needing more development and integration with the Rhizobia research; d) low availability and high prices of inputs such as lime and fertilizers. However, due to the limitations in the time available for assesssment of the situation, these points have to be submitted to a better analysis. Another important aspect is that a strong trend seems to exist there toward the integration of efforts among researchers, as is evidenced by the rhizobiologists meetings promoted by Prof. Susano Saono of the Lembaga Nasional Institut.

RECOMMENDATIONS FOR THE ESTABLISHMENT OF A PROGRAM ON PRODUCTION OF Rhizobium INOCULANTS FOR LEGUMES

For the establishment of a sound program on production of inoculants, the following main factors are considered important:

1. Availability of efficient and competitive Rhizobium strains for the desired legumes.
2. Existence of a trained microbiologist for the inoculant production process in itself, maintenance of the culture collection and quality control of inoculants and research for selection of strains.
3. Integrated team at IPB consisting of the above mentioned microbiologist, agronomists, and soil fertility specialists. The integrated work with plant physiologists and breeders would also be recommended.

4. Establishment of a simple but reliable laboratory and greenhouse infrastructure for the isolation of Rhizobia strains, maintenance of well-cared for culture collection, quality control of inoculants and selection of strains.
5. Establishment of facilities for inoculant production. The existence of a high quality, laboratory made inoculant produced at a governmental or university body is also important for the initiation of a program, and for providing inoculant for use at experimental sites, demonstrations, etc. It would also provide a standard for comparison with commercial inoculants.
6. Enrich bibliography availability with more important books on biological nitrogen fixation and journals related to Rhizobium research such as Plant and Soil, Agronomy Journal, Crop Science, Plant Physiology, Soil Sci. Soc. Amer. J., Trop. Agric., Austr. J. Agric. Res. (in English), Pesquisa Agropecuaria Brasileira and Revista Brasileira de Ciência do Solo (in Portuguese).
7. Strengthening of the cooperation between researchers, extensionists, and legume promotion programs by means of jointly sponsored seminars, workshops and field days by supplying inoculants for demonstrations, and by jointly sponsoring information and research projects, technical advising, etc.

8. Strengthening of the interdisciplinary cooperation between the rhizobiologists and other fields of research, e.g. supplying inoculants and technical assistance for nodulation evaluation at variety trials, soil fertility experiments, etc. Integrated research work is certainly cheaper, more efficient and more productive than isolated work.
9. Establishment of an integrated cooperative research program involving researchers and institutions in the area of Bogor.
10. Establishment of sound research priorities for the integrated program based mainly on: a) gathering of efficient, competitive Rhizobium strains with high survival capacity in different environmental conditions for legumes of economic importance and responsive to inoculation; b) evaluation of soil limiting factors to nodulation and N_2 fixation, especially in the areas where legumes of economic importance such as soybeans are being promoted.
11. Establishment of opportunities for key elements of the IPB program to visit other Rhizobium research centers. Due to the similarity of soil limiting factors in Brazil and Indonesia and the philosophy of the extension-minded Rhizobium program at the Porto Alegre MIRCEN, we suggest the visit of Dr. Fred Rumawas (soil fertility specialist) and Agnes Rumawas (microbiologist) to Brazil for short-term training.

12. Establishment of connections with other institutions in the region such as the Bangkok MIRCEN and CSIRO in Australia. The NifTAL MIRCEN at Hawaii is also an excellent center for Rhizobia strains, bibliography, information and training. Of course, Port Alegre-MIRCEN, in spite of the distance, will also cooperate with the Indonesian program in the above aspects.

ANNEX NO. 1

FIRST SEMINAR - JUNE 22, 1982

THE USE OF THE *Rhizobium* TECHNOLOGY

by

J. R. Jardim Freire*

Legumes have been an important part of the diet of people everywhere in the world since ancient times. They are part of many agricultural production systems at the subsistence level as well as in systems of agriculture using high levels of technology. The role of legumes in the improvement of soil fertility was recognized by the Romans. The role played by bacteria and nodules in the fixation of atmospheric nitrogen has been well known for one hundred years. The technology to take advantage of this symbioses is available everywhere. However, in relation to its potential, little use has been made of nodulating legumes and little concern exists, even among agronomists, for the adequate exploitation of the potential of symbiotic dinitrogen fixation. There is too little concern for wide-spread use of inoculation practices, especially in tropical areas. Extensionists and farmers in most regions have not developed the practice of checking nodulation as an indirect way to evaluate the supply of nitrogen for their crops. It is usually forgotten that in addition to the nitrogen in the soil, nodulated legumes have the potential for obtaining nitrogen from the air. In order to attain the benefits of symbiosis, consideration must be given to the close interrelationship between the plant, the bacteria and the environment.

*Associate Professor-Microbiology, Department of Soils, Federal University of Rio Grande do Sul, Porto Alegre, BRAZIL.

The effective use of Rhizobium/legume symbiosis must involve the work of an interdisciplinary, applied research team, an appropriate program for legume promotion, appropriate linkages between research and extension and well-trained extensionists. The research must be primarily directed toward the search for locally adapted, efficient Rhizobia strains, the evaluation of the limiting factors in N_2 fixation, and selection of legume cultivars with high symbiotic capacity.

As a starting point, the production of high quality inoculants in the laboratory is essential for the estbalishment of a program. At the same time, it is necessary to maintain services for control of the quality of the inoculants available. Nothing can damage the expansion of the practice of legume inoculation more than poor quality inoculants on the market. The work of researchers in gathering efficient strains is useless if these strains are not used for the production of the inoculants. The work of gathering and spreading information on soil limiting factors is also useless and farmers will waste their other inputs such as lime and P, K fertilizers if the supply of N is deficient due to a bad inoculant.

Final recommendations to start a Rhizobium program:

1. Form an ample culture collection of strains of imported or local origin for legumes of economic or potential economic importance.
2. Establish priorities in relation to the need of inoculation and legumes.
3. Establish an objective program for strain selection, aiming mainly at efficiency of N_2 fixation, competitiveness for nodule sites and survival in the soil. An integrated program, involving breeders, agronomic researchers, forage specialists, etc. is necessary for best utilization of available resources.
4. Identify and evaluate the soil limiting factors such as pH, major and minor nutrient deficiencies and toxicity problems, in cooperation with soil specialists.

5. Produce inoculants to meet the needs of farmers and for experimental use at other research areas and demonstrations conducted in close cooperation with extension agents and agronomic researchers.
6. Establish services for informal quality control of inoculants. At a higher level, formal control, supported by special law will be useful.
7. Establish a program for adequate training of personnel in agriculture schools, and train agronomists and extensionists in the field by means of short-courses, seminars, workshops, etc.

ANNEX NO. 2

SECOND SEMINAR - June 28, 1982

ESTABLISHMENT OF A PROGRAM AND LIMITING FACTORS IN THE APPLICATION OF THE *Rhizobium* TECHNOLOGY

by

J. R. Jardim Freire*

A. Establishment of a Program

The implementation of a program for the attainment of higher benefits from the N_2 fixation by the symbiosis *Rhizobium*/legumes must be based on:

1. General assessment of the situation.

   1.1. Important legumes and their potential - grain, forage, green manure, reforestation, soil covering; for food and for the economy of the country.

   1.2. Research - existing institutions, personnel, facilities, bibliography availability, work underway on *Rhizobium*.

   1.3. Limiting factors - soil, credit, seeds, inputs (lime, fertilizers, etc.). Infrastructure.

   1.4. Legume promotion programs.

   1.5. Inoculants - availability, production, quality control.

   1.6. Agrarian structure - farmers, education, size of property, etc.

   1.7. Extension - linkages with research. Training on *Rhizobium* and legume inoculation.

*Associate Professor, Department of Soils, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

2. *Rhizobium* promotion program

2.1. Research - establishment of priorities

Integrated program for an inter-institutional and inter-disciplinary team efforts.

2.2. Establishment of a program for inoculant production and quality control.

2.3. Program for coordination with extension services and legume promotion programs by other organizations or departments. Training of extensionists. Program for demonstration plots under responsibility of the extensionists. Information papers and bulletins for the extensionists. Workshops and seminars.

2.4. Assumptions and recommendations for the success of the program availability of inputs-lime, fertilizers. Credit, etc.

2.5. Establishment of goals and deadlines by the integrated research/extension

Situation Now	Situation expected or goals in	Means	Assumptions

B. Constraints

In the evaluation or assessment of the situation the following factors can be found:

1. In the research activity:
   - 1.1. Inadequate training of the researchers - microbiologists, soil specialists, legume breeders, etc.
   - 1.2. Badly established priorities and/or insufficient applied research.
   - 1.3. Researcher isolation.
   - 1.4. Deficient interdisciplinarity.
   - 1.5. Deficient promotion of the research work.

2. Inoculants
   - 2.1. Poor quality of available inoculants in terms of number of cells and use of strains adapted to the crop varieties grown and to local environment.
   - 2.2. Prices too high.
   - 2.3. Deficient or nonexistant quality control.

3. Extension
   - 3.1. Nonexistence or deficient legume promotion programs.
   - 3.2. Poor connection with research.
   - 3.3. Poor training on *Rhizobium*.

4. Inputs, credit, infrastructure deficiencies.

C. Microbiology Resources Center (MIRCEN) activity in Porto Alegre, Brazil

Objectives of the agreement between UNEP/UNESCO/ICRO and the Brazilian Government. Activities in training, research, culture collection and dissemination of strains and information.

ANNEX NO. 3

TRAINEE ASSIGNMENT - First Part*

DEVELOMENT OF A *Rhizobium* PROMOTION PROGRAM

INTRODUCTION

During the first two Five-Year Development Plans, the Government of Indonesia made efforts to increase its rice production to top priority. These efforts have paid off and at the end of the current Third Five-Year Development Plant, rice production has reached sufficiency levels, so that more attention can be paid to other crops. These crops are collctively known as the palawija crops, made up of cereals, tuber crops and grain legumes.

This new emphasis creates new demands for production technology for palawija crops. Grain legumes will be a major group of crops worth improving. One of the major problems is increasing the low yields and another is the introduction of these crops in agricultural systems in newly cleared land.

Chemical, biological, physical, capital, human, infrastructural and institutional inputs have to be considered. One of the biological inputs, which also happens to save energy, is the introduction of effective *Rhizobium* spp. It is known that the *Rhizobium*/legumes combinations benefit man.

Rhizobium is also used extensively in pastures, with legume-grass combinations. Leguminous cover crops could be improved by the introduction of this bacterium.

*Second part (Conclusions) will be completed by the group of trainees, as it was in the first part.

CURRENT SITUATION

1. Legumes

The most important grain legumes are soybeans, peanuts, and mungbeans. These three species are grown widely, although only the first two are mentioned (Table 1). Yields are still low (0.7-0.8 ton/ha for soybeans and 0.8 ton/ha for peanuts).

Less important edible legumes are common beans (Phaseolus vulgaris), cowpea (Vigna.sinensis), broad bean (Ph. lunatus), sword bean (Canavalia ensiformis), winged bean (Psophocarphus tetragonolubus), Dolichos lab-lab, pigeon pea (Cajanus indicus), Voandzeia subterranea, pea (Pisum sativum, Phaseolus calcaratus), Mucuna utilis a vegetable tuber, and the yam bean (Pachyrrhizus erosus). Four leguminous tree species which produce edible seed are Parkia speciosa, Pithecolobium lobatum, Leucaena glauca and to a lesser extent, Adenanthera pavonina.

Important pasture legumes are Stylosanthes gracilis and Styl. humilis adapted to acid soils and Macroptilium atropurpureum for dry areas.

Green manures and cover crops are often used. the most common ones are Crotalaria spp. Tephrosia spp. Flemigia contorta, Pueraria javanicum, Calopogonium muncunoides, Cal. cearuleum, Centrosema pubescens and Psophocarpus palustri.

Yam bean seed, Tephrrosia leaves and certainly Derris eliptica countain rotenon or rotenoids, which hold promise as insecticides. Indigofera has been widely used as a source of the dye indigo, before the introduction of synthetic dyes.

2. Research

Research with legumes and Rhizobium or N-fixation in general are scattered in various institutions and not yet coordinated. The main ones are the National Biological Institute, research institutions under the Department of Agriculture and universities which are under the Department of Education and culture.

The research effort is not yet focused on specific and national priorities because of past preoccupation with rice. Most institutions have laboratory facilities for general microbiological research and green houses. Some have access to experimental farm facilities.

Facilities available, such as at the Institut Pertanian Bogor, include laboratories with the basic equipment for Rhizobium research and production, greenhouses and field facilities in Bogor as well as in Sumatra and Kalimantan (mineral and muck soils). This could be expanded greatly by cooperation with several research institutions (food crops, soil, animal husbandry, forestry, and industrial crops).

Table 2 gives an idea of research conducted in recent years.

3. Limiting factors

It is well understood that a Rhizobium promotion program must be part of an integrated legume improvement program within a given cropping system. The limiting factors, therefore, are those related directly to legume production.

a. Soil

The low yields shown in Table 1 are in part caused by poor soil conditions. In the most important soybean production area, Eastern Java, soybeans are grown after rice without proper soil preparation. Poor drainage becomes a serious limiting factor.

Land available for extending legume production is dominated by ultisols and oxisols. These soils are characterized by low pH, low P, and moderate to high Al, Fe and Mn levels. Liming the soil to neutralize the adverse effects of the last three elements mentioned is important. This practice is not common in Indonesia. The slightly higher yield of peanuts is probably due to the fact that this species tolerates higher levels of Al. Soybeans, along with corn and sorghum are susceptible to Al toxicity.

After liming, the Rhizobium/legume combination will respond to P and K applications. The main problem here is the common practice to use

small amounts of fertilizers, instead of high initial dosages for corrective fertilization (250-500 kg TSP/ha). This is probably caused by the failure of researchers to recognize limits set by micro-nutrient deficiencies (Cu, Zn, B), since they will be in greater demand, while only Mo is made more available by liming. Zinc availability is further reduced by high P applications.

In many of the above-mentioned soils, Mg is also low. Addition of calcitic lime will create a Ca-Mg imbalance. To achieve the ideal 3:1 Ca to Mg ratio, dolomitic lime would be more preferable, since Kieserite is an expensive imported fertilizer.

The agricultural lime must be fine enough to be useful. The finer the material, the higher the neutralizing value but it is also more expensive to produce. Limestone crushed to pass 60-mesh is considerered satisfactory in most cases.

b. Credit

Credit to improve rice production has shown its benefits. For grain legumes this program has not been fully realized. Only a small short-term credit program exists to cover purchase of seed, fertilizers, and pesticides. Long-term credit to enable lime applications and corrective fertilization does not exist.

Two important institutions through which credit could be channeled are the rural banks and the cooperatives. The reluctance to extend long-term agricultural credit to small holders is primarily caused by the uncertainity of repayment of the loan.

c. Seed

Another major limiting factor is the timely supply of high quality seed of superior cultivars. Improved varieties of soybean, peanuts, mungbeans, and cowpeas are available at research centers and are used in field trials. Farmers, however seldom use them. This may be due to ignorance, higher input levels needed, or the unavailability of seed.

Air tight containers with burned lime as a dessicant is a cheap and effective method of seed storage. Leguminous seed can be stored this way for at least one year. The most ideal situation is if cooperatives or the private sector could be mobilized.

4. Legume promotion program

Legumes are already incorporated in Indonesian agriculture and their usefulness is generally understood. The annual consumption of soybeans, peanuts, and mungbeans between 1974 and 1977 was respectively 4.4, 2.67 and 9.54 kg/capita. Between 1961 and 1970, the daily plant protein consumption was 34.4 g/capita, of which only 18 percent was of leguminous origin. Cereals supplied 63 percent of the plant proteins in the diet.

Their figures indicate that the consumption of grain legumes could be increased. The over-dependence on cereals (mainly rice) is both traditional and a matter of low income levels. Economic improvement is already felt as an increase in demand for grain legumes.

A palawija pilot project sponsored by the USAID will be in operation soon. It is anticipated that through this integrated effort yields can be increased and farmers income improved. A Rhizobium promotion program would fit into this project perfectly.

5. Inoculants

Until recently, inoculants were imported. Very good results have been obtained with Nitragin on improved soils. Up to 120 kg N/ha of fixed nitrogen have been reported on soybeans.

These results have triggered efforts to produce the inoculants. The Gajah Mada University is marketing their product (Legin) and RISPA (Medan) has been experimenting with inoculant production for cover crops. By the end of this year, the Institut Pertanian Bogor will be producing inoculants for their experiments in West Sumatra and Jambi.

Laws to ensure quality control of inoculants do not exist. This weakness could be remedied; meanwhile, the producers have to control the quality of their produce voluntarily. This is particularly important because good results will greatly influence the acceptance of this new input by the farmers.

Table 1. Harvested area, Production, and Yield of Peanuts and Soybeans in Indonesia 1975-1976

	PEANUTS						SOYBEANS					
	Hvstd. Area/Ha		Production (Ton)		Yield 100 kg/ha		Hvstd. Area/Ha		Production (Ton)		Yield 100 kg/ha	
	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976
1	2	3	4	5	6	7	8	9	10	11	12	13
1. DKI JAKARTA	127	148	111	133	8.74	9.02	--	--	--	--	--	--
2. JAVA BARAT	77,612	72,910	62,421	61,974	8.04	8.50	28,339	28,681	20,901	20,249	7.38	7.06
3. JAVA TENGAH	104,156	90,542	82,286	75,512	7.90	8.34	147,716	102,247	108,978	72,493	7.38	7.09
4. D. I. YOGYAKARTA	30,901	24,408	25,523	19,975	8.26	8.11	31,262	26,745	21,189	20,273	6.78	7.58
5. JAWA TIMUR	159,758	126,501	126,448	97,532	7.91	7.71	393,763	340,887	316,593	267,255	8.04	7.84
JAWA & MADURA	372,554	314,509	296,789	254,532	7.97	8.11	601,080	498,560	467,661	380,270	7.78	7.63
6. D. I. ACEH	3,053	4,040	2,461	3,931	8.06	9.73	3,561	3,886	2,586	2,582	7.25	7.24
7. SUMATERA UTARA	13,033	14,358	15,131	14,143	11.61	9.85	9,961	9,903	8,317	7,606	8.35	7.68
8. SUMATERA BARAT	3,727	3,780	4,219	4,426	11.32	11.71	3,350	2,936	3,491	3,183	10.42	10.84
9. RIAU	599	565	474	435	7.92	7.70	194	187	125	124	6.46	6.65
10. JAMBI	637	641	646	599	10.14	9.35	536	534	579	580	10.82	10.86
11. SUMATERA SELATAN	2,981	3,003	2,355	2,806	7.90	9.35	3,714	3,006	2,930	2,955	7.89	9.83
12. BENGKULU	436	590	312	508	7.16	8.61	224	378	144	249	6.42	6.59
13. BAMPUNG	6,942	5,448	4,852	3,695	6.99	6.79	36,573	30,737	35,110	25,512	9.60	8.30
SUMATERA	31,408	32,425	30,450	30,549	9.69	9.42	58,113	51,567	53,278	43,022	9.17	8.34
14. KALIMANTAN BARAT	400	361	332	249	8.31	6.90	2,574	2,561	1,683	1,795	6.54	7.01
15. KAL. TENGAH	209	211	136	137	6.50	6.51	--	---	---	---	--	--
16. KAL. SELATAN	2,005	4,284	1,835	3,920	9.15	9.15	362	927	259	513	7.16	5.53
17. KAL. TIMUR	252	238	154	141	6.12	5.92	244	243	173	174	7.10	7.17
KALIMANTAN	2,866	5,094	2,457	4,447	8.57	8.73	3,180	3,731	2,115	2,482	6.65	6.65

Table I. (Cont.)

	PEANUTS						SOYBEANS					
	Hvstd. Area/Ha		Production (Ton)		Yield 100 kg/ha		Hvstd. Area/Ha		Production (Ton)		Yield 100 kg/ha	
	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976	1975	1976
1	2	3	4	5	6	7	8	9	10	11	12	13
18. SULAWESI UTARA	4,178	3,966	3,510	3,046	8.40	7.68	1,750	1,683	1,346	1,128	6.69	6.70
19. SUL. TENGAH	3,548	3,351	1,969	1,840	5.55	5.49	965	960	593	607	6.25	6.32
20. SUL. SELATAN	32,258	22,659	24,194	13,369	7.50	5.90	7,478	7,441	4,980	4,956	6.66	6.66
21. SUL. TENGGARA	850	773	655	554	7.70	7.17	531	1,276	243	800	4.58	6.27
SULAWESI	40,834	30,749	30,328	18,809	7.42	6.12	10,724	11,360	7,162	7,491	6.68	6.59
22. MALUKU	2,405	2,484	1,347	1,535	6.60	6.18	175	170	106	100	6.05	5.88
23. IRIAN JAYA	1,184	1,223	742	744	6.27	6.08	157	152	121	120	7.71	7.89
MALUKU DAN/ IRIAN JAYA	3,589	3,707	2,089	2,279	5.82	6.15	332	322	227	220	6.84	6.83
24. BALI	8,625	8,050	7,262	8,436	8.42	10.48	13,527	10,974	13,557	9,789	10.17	8.92
25. NUSA TENGGARA BARAT	9,107	11,120	7,440	9,815	8.17	8.83	64,447	59,432	45,500	38,571	7.06	6.49
26. NUSA TENGARRA TIMUR	5,536	5,228	2,868	2,745	5.18	5.25	286	289	131	136	--	--
BALI & NUSA TENGGARA	23,268	24,398	17,570	21,000	7.55	8.61	78,260	70,695	59,386	48,496	7.59	6.86
TOTAL OUTER JAVA	101,965	96,373	82,894	77,064	8.13	8.00	150,609	137,675	122,170	101,711	8.11	7.39
INDONESIA	474,519	410,862	379,683	332,030	8.00	8.08	751,689	636,235	589,831	481,981	7.85	7.58

Table 2. Research on Biological N-fixation in Indonesia

INSTITUTION	PROJECT LEADER	PROJECT TITLE	LIMITING FACTORS
1. FIPPA-UNPAD BANDUNG Pajajaran Univ.	Ahmad Joenoes	1. The use of inoculant to increase the production of Crotalaria juncea L. and soil productivity	
		2. The inoculation of Rhizobium on Calopogonium cearuleum cuttings to increase seed production.	
	Razali Usman	3. The use of simple empirical seeding for the isolation and cultivation of Rhizobium sp.	budget, research facilities
		4. The use of mixed inoculant on grain legumes.	
2. The faculty of Veterinary Med. & Animal Husbandry Udayana Univ. Denpasar-Bali	I. M. Nitis	5. Tropical legumes as companion crops for food crops.	
	Oka Kartika	6. Evaluation of nodulation on tree legumes.	
	I. Ketut Lana & I. Gst. Md Oka Nurjaya	7. Intercropping of corn with stylo.	budget
3. Bogor Research Institute for Food crops (BORIF), Bogor	S. Sumaatmadja	8. Research on Rhizobium for soybean.	
	D. Pasaribu	9. Interaction of Rhizobium inoculation and N fertilization on peanuts.	
		10. The effect of fertilization and Rhizobium inoculation on the growth and production of peanuts.	
		11. Rhizobium inoculation on mungbean.	
		12. The effect of inoculation with R. japonicum, N and P fertilization on the growth and production of soybean.	research staff
		13. The effect of various inoculants on several varieties of soybean.	
	Rasti Saraswati & Ratna S. Hadioetomo	14. The collection of Rhizobium strains from soybean producing areas in East Java.	budget, research facilities, research staff & technicians

Table 2. (Cont.)

INSTITUTION	PROJECT LEADER	PROJECT TITLE	LIMITING FACTORS
	S. Partohardjono & H. Vergilius	15. The effect of inoculation with Azolla on the growth and yield of rice and the effect of its residues on the following crop.	
		16. The effect of micronutrients on Azolla and how if effects the growth and yield of rice.	
	S. Partohardjono, B. T. Gumala & M. Bastaman	17. The effect of spacing on the development of Azolla in relation to the inoculation time and the application of chemical N fertilizer on the growth and yield of rice.	
4. Sukamandi Res. Inst. for Food Crops	O. O. Hidayat, H. Anwarhan & Pramono Djojodarmodjo	18. The response of local varieties of soybean & peanut on the inoculation with selected Rhizobium strains from Niftal/Univ. of Hawaii.	
5. Research Institut for Animal Husbandry	M. E. Siregar	19. The ability of root nodules of four pasture legumes to survive under seasonal flooding.	
6.	M. Siahaan	20. Rhizobium inoculation on the cover crops Calopogonium caeruleum.	
7. The Fac. of Agr. Gadjah Mada Univ.	Jutono	21. The production of R. japonicum inoculant using the biphasic system.	
		22. The isolation and evaluation of the ability of R. japonicum isolates on the botanical composition of soybean.	
		23. Azotobacter spp. as N_2 fixer & the production of biostimulants.	
		24. The application of Rhizobium inoculant (Legin) on the transmigration areas in Indonesia.	
	Siti Kabirun	25. Double symbiosis is on soybean and its effect on seed produbtion	
	S. Kabirun & John Bako Baon	26. Vesicular-arbrescular mycorrhiza on soybean.	

Table 2. (Cont.)

INSTITUTION	PROJECT LEADER	PROJECT TITLE	LIMITING FACTORS
	J. Soedarsono	27. The association of Gramineae with N-fixing microorganisms.	
8. National Biology Institut, Bogor	Susano Saono and Herry Karsono	28. The effect of inoculation with Rhizobium spp. on the growth of Vigna umbellata and V. unguiculata.	
	Susano Saono, M. Sudjana, H. Karsono	29. The production of inoculant with peat as the carrier.	
	Susano Saono & M. Sudjana	30. The storage of microorganisms by freeze-drying.	
	S. H. A. Lubis and S. E. Pratinyo	31. The evaluation of germ plasma from Cajanus cajan.	
	Sumadi Atmowidjojo	32. Seed production from the first and second generation of Phophocarpus tetragonolobus.	
	S. Atmowidjojo & M. H.Siagian	33. Seed production of several collections of Lablab purpurea.	
	Harmastini, S. Abdulkadir & S. Saono	34. The distribution of N-fixing Azolla and blue green algae in irrigated rice field.	
9. Soil Research Institut, Bogor	M. Sudjadi Mursidi, Tini Prihatini & J. Sri	35. The effect of fertilizer and environment on the growth of Azolla pinnata.	Research staff & technicians
	Adiningsih S.	36. The effect of fertilizing Azolla on the growth and production of rice.	
	H. Karsono, Tini Prihatini, S. Saono, J. Sri Adiningsih S., M. Sudjadi & Mursidi S. ME.	37. The effect of several Rhizobium inoculations on the growth and production of grain legumes.	
	M. Sudjadi, Mursidi, T. Prihatini, & J. Sri Adiningsih S.	38. The effect of liming, P and micronutrient fertilization on nodulation and production of grain legumes on the red yellow podzolic soil of Sitiung.	Research staff & technicians.

Table 2. (Cont.)

INSTITUTION	PROJECT LEADER	PROJECT TITLE	LIMITING FACTORS
		39. The effect of several grain legumes (cover crops) on soil fertility (chemical & physical) of yellow podzolic in Sitiung.	
10. The Fac. of Agr. IPB, Bogor	R. S. Hadioetomo T. Imas AH	40. The selection of superior Rhizobium strains for peanuts	Research staff & technician's budget, research facilities.
11. The Fac. of Agr. Univ. of Pajajaran Bandung	Dedeh H. Arief	41. The distribution of blue green algae in irrigated rice fields of various altitudes and soil types in West Java.	
12. The Fac. of Agr. IPB, Bogor	S. Surowinoto	42. The response of Clark 63 normal Soybean and Clarkrji soybeans to nitrogen fertilization (MS. thesis, IPB. Bogor, 1977.	
13. The Fac. of Agr. IPB, Bogor	S. Surowinoto, E. Sjamsuddin and A. Chozin	43. The influence of Rhizobium japonicum to yield and yield components of soybeans at high levels of nitrogen fertilization (P_4-IPB).	
14. The Fac. of Agr. IPB, Bogor	D. Prasastyawaty	44. The development of Rhizobium japonicum nodules on soybeans. Special problem, (IPB. Bogor).	
15. The Fac. of Agr. IPB, Bogor	Z. Mahnud	45. Nitrogen transfer from Rhizobium japonicum nodules. (MS. thesis, IPB. Bogor, 1979).	