

On-farm Trials of Biological Nitrogen Fixation (BNF) Technology on Beans (*Phaseolus Vulgaris*) in Mountain Province

David Y. Fomeg-As¹, Tessie M. Merestela²,
Jose G. Balaoing², Maria Luz D. Fang-asan²

Keywords: BNF technology, lime, inoculum, inoculant, *rhizobium*, biological nitrogen fixation, dinitrogen fixation, soil test kit, farmer's practice, nodulation

ABSTRACT

On-farm trials of biological nitrogen fixation (BNF) technology were conducted in Mountain Province with the aims of assessing the need for inoculation of beans (*Phaseolus vulgaris*) in farmers' fields, and comparing the BNF technology with other agricultural practices in the cultivation of beans (*Phaseolus vulgaris*) as to their effects on nodulation and yield.

Three (3) farmer cooperators in Mountain Province were identified and their farms were utilized for farm trials of this study. The farm sites were in Egan and Amgayang, Tadian and Ambasing, Sagada. In each farm, the field trials were laid out following the randomized complete block design (RCBD).

Inoculated beans showed higher nodulation as compared to the uninoculated beans. Nodulation of beans on farmer's practice either limed or unlimed were also generally lower than the other practices. However, result indicated that there was a significant positive response to inoculation in Egan, Tadian; which is most likely due to sparse population of indigenous *rhizobium* in the soil. This result implies, on the other hand, that indigenous population of *Rhizobium* in Amgayang and Ambasing farms can compete with the commercial strain used for inoculation. Bean inoculation is thus required in Egan farm but not necessarily required both in Amgayang and Ambasing farms.

Yield of beans on the first harvest of inoculated treatments were generally lower than the other treatments including the farmer's practice, but were comparable since differences were not significant.

¹ Mountain Province State Polytechnic College, Bontoc, Mountain Province

² Benguet State University, La Trinidad, Benguet

The number of nodules and yield of beans were found to be linearly related based on simple regression and correlation analyses made. The correlation though was negative; which would imply the influence of some other factors other than the nodules such as treatment effects on yield.

INTRODUCTION

Background of the Study

Beans are commonly cultivated in some places of Mountain Province. Some bean growers usually produce beans for market while others cultivate them both for market and home consumption. In light of the new trends on organic agriculture nowadays, the minimal use of inorganic fertilizers in the fertilization of crops is of importance. This can be done directly with the use of existing technology on biological nitrogen fixation in the case of legume crops like beans, or indirectly using the same technology in the fertilization of other crops either by using legumes as green manure or rotation crop.

Symbiotic nitrogen fixation in legumes is known since the 18th century. This mechanism is therefore not new in the scientific world. Yet, until today, very few farmers know the technology. The dissemination of the technology to farmers is deemed necessary. However, environmental variations (both microclimate and macroclimate) may adversely affect the performance of the technology. Moreover, indigenous populations of the microorganisms (*Rhizobium*) may already be enough. In such case, the legume may not respond positively to legume inoculation (BNF technology) with the desired *Rhizobium* species.

Rhizobium is any of a genus (*Rhizobium*) of small heterotrophic soil bacteria capable of forming symbiotic nodules on the roots of leguminous plants and of there becoming bacteroids that fix atmospheric nitrogen (INFOPEdia, 1995). It is chemoorganotrophic, utilizing a wide range of carbohydrates and salts or organic acids as carbon source (Trinidad and Yoshida, 1989).

In their study, Merestela, et al. (1996), found that populations of *Rhizobium leguminosarum* biovar *phaseoli* and *viceae* in the soil science experiment station is sparse and few. Sparse population of indigenous *rhizobium* results to low biological nitrogen fixation, more so when the strains present are ineffective. This was evident in the positive response of legumes to inoculation in the station.

Other fields too, may have low counts of *rhizobium* and need inoculation. Dart (1974) mentioned that the distribution of *rhizobium* in soils can vary even within one field and numbers may decline when legumes are not grown. Field experiments of Slattery, et al. (n. d.) showed that on some soil types rhizobial strains were present that are capable of infecting the host legume and forming nodules, but at others *rhizobia* were not present in the soil and no nodules are formed.

Inoculation is beneficial in two ways. First, it improves nodulation and N₂-fixation; second, it may increase *rhizobium* population in the soil. Higher number or population of *rhizobium* increases nodulation rate; therefore may also increase N₂-fixation rate. Generally, legume yields are increased through N₂-fixation.

Legume crops however, do not always respond positively to inoculation. Lack of response may be because of adequate natural nodulation—the applied inoculum does not become established through failure to survive or colonize (. . . the root hairs), or compete with indigenous *rhizobia*; or there are conditions unfavorable for nodule formation and functioning (moisture, temperature, nutrient deficiency, combined N) (Vincent, 1970).

Moreover, the indigenous *rhizobium* or applied inoculum (*Rhizobium* strains) may have poor N₂-fixing effectiveness and symbiotic effectiveness. Field trials assess the effectivity or infectivity of *rhizobium* and the need for inoculation.

Thus, the general objective of the study was to conduct on-farm trials of biological nitrogen fixation (BNF) technology on beans in Mountain Province. The farms are located in Ambasing, Sagada; and Amgayang and Egan, Tadian.

Objectives of the Study

Specifically, the objectives of the study are:

(1) to assess the need for bean inoculation (BNF technology in beans) in selected farms of Mountain Province, and

(2) to compare BNF technology with other agricultural practices in the cultivation of beans (*Phaseolus vulgaris*) as to their effects on yield and nodulation.

Importance of the Study

The technology on legume inoculation or leguminous symbiotic/biological nitrogen fixation (BNF) is not yet widely disseminated to farmers. Almost no farmers in the Cordillera are informed on the use and purpose of legume inoculation. The absence of a market center (market access constraints) for microbial inoculants may have aggravated the situation. Consequently, farmers still apply nitrogen fertilizers for legume crops, though some does not use any type of fertilizer.

Nitrogen fertilization of legume crops though is not advisable, except in the early stage of growth (Finck, 1982). Legumes undergo N_2 -fixation when in symbiosis with *rhizobium*, thereby atmospheric N is utilized for their growth and development. Farmers are therefore adding much input for fertilizers especially on nitrogen-fertilizers, which is to say a waste of money. N_2 -fixation, however, may not be effective when populations of the desired *rhizobium* in the soil is sparse and few. Consequently, N_2 -fixation will not be able to supply sufficiently the needed nitrogen by the crop throughout its growth and development.

Legume inoculations using specific *Rhizobium* strains enhance N_2 -fixation, especially in soils with sparse and few *rhizobium* populations. Inoculation may even increase natural populations of *rhizobium* when introduced strains become established.

Applied inoculum in the soil usually fails to survive or colonize and compete with indigenous *rhizobia* due to variable soil environment conditions. Hence, farmers may need to inoculate every time they grow legumes especially when population of indigenous *rhizobium* in their field is ineffective or sparse and few.

Anent to this, it is advisable to do field trials to assess the need for legume inoculation and the effectiveness of introduced strains including the indigenous *rhizobium*. More on-farm trials on legume inoculation are needed—due to variable soil environment conditions, to transfer the technology to farmers, and to improve farmer's vegetable and grain legume production.

Place and Time of the Study

Farm trials were done in Ambasing, Sagada; Amgayang, Tadian; and Egan, Tadian. All farms are located in Mountain Province. Liming was done three months earlier in mid-October 1999 prior to sowing in late January 2000. The crop was allowed to grow until May 2000.

MATERIALS AND METHODS

Farm Sites and Farmer Cooperators

Farmer cooperators, who cultivate beans, were identified in selected places of Mountain Province. Selection was according to their willingness to see for themselves the performance of BNF technology and allow the use of a parcel of their farm, and availability of the land for cultivation during the duration of the study.

They were Mrs. Lilian T. Banglo and Elordes R. Kidicdian of Egan and Amgayang, Tadian, respectively; and Mrs. Carmen M. Pomeg-as of Ambasing, Sagada. Field trials were done at their respective farms to determine the performance of biological nitrogen fixation (BNF) technology on beans (*Phaseolus vulgaris*) in situ.

Tadian and Sagada have a semi-temperate climate throughout the year, though the latter is generally cooler possibly due to higher elevation and more trees. In addition, Tadian and Sagada have a climatic condition that approximates that of La Trinidad and Club John Hay, Baguio City, correspondingly.

Procedure and Data Analysis

The farmer cooperators helped maintained the farms. A standard microbial inoculant (*Rhizobium*,) in a commercial package known as Legumin was obtained from BIOTECH, UPLB, Los Baños, Laguna for use in the trials.

Before sowing, the garden plots were labeled with treatments. Randomized blocking, though, was impossible due to variable orientation and dimension of the lots in each farmer's field and among the three farms. The possibility of a combined experiment, whereby combined analysis of each experiment is possible if conducted simultaneously in places more or less at random over an area and employs the same design and treatments, was therefore ruled out.

Nevertheless, two to three replicates were assigned and laid-out accordingly with the treatments randomly distributed following inasmuch as possible the randomized complete block design despite the orientation and limited space of the lot. This was done for the purpose of statistical comparison of the treatments. The dimensions of the plots used were 0.5 m X 5.0 m or close to this in each field.

Since one value was missing on the data of yield for Egan, the analysis of covariance (ANOCOVA) for estimating missing data in a randomized complete block design was used. The analysis of variance (ANOVA) was used for the rest of the data. Comparison of means was done with the Duncan's Multiple Range Test (DMRT). A simple linear regression and correlation analysis was also made to determine the relation between the number of nodules and yield of beans.

The treatments were as follows:

- T₀ – Farmer's Practice, FP (unlimed), control
- T₁ – Farmers' Practice, FP (limed)
- T₂ – BNF
- T₃ – BNF + FP
- T₄ – BNF + Bean Fertilizer Requirement
- T₅ – Soil Test Kit Analysis (STK)
- T₆ – Bean Fertilizer Requirement (BFR)

Only the treatments with BNF and T₁ were limed. This was done as part of the BNF technology to make sure that the soil pH is adjusted to near neutral favorable to the *Rhizobium*; and to make a comparison between limed and unlimed plots following farmer's practice. The amount of liming material used (agricultural lime, CaCO₃) was determined with the La Motte soil test kit on pH using an air-dried composite soil sample. Liming was done simultaneously at the farms three months in advance prior to sowing. Sowing was done for all treatments in the three farms simultaneously. The seed used was a pole kidney bean (Benguet bean) of Alno cultivated variety. Inoculation was done by coating the moistened seed with enough amount of the inoculant (1 tbsp. inoculant/kg seed).

The farmer's practice (FP) is the application of an estimated one-tablespoon Triple-14 inorganic fertilizer per hill (personal interview and communication with the farmer cooperators), while the bean fertilizer requirement (BFR) is simply the recommended rate for bean, which is 30-100-40 kg NP₂O₅K₂O/ha (Bautista and Mabesa, 1986). On the contrary, Treatment 5 was based on La Motte soil test kit analyses on nitrogen, phosphorus, and potassium with the use of an air-dried composite soil sample; thus, the amount of fertilizer material might vary for each farm. All fertilizer materials used except for solophos (0-18-0) were applied as sidedress.

The primary basis of the performance of the BNF technology was determined from the responses of the crops to inoculation in terms of nodulation and yield. The crop's response was compared between treatments.

RESULTS AND DISCUSSION

Soil tests. Qualitative soil test analyses on pH, nitrogen, phosphorus, and potassium (potash) with the LaMotte soil test kit are shown on the following tables. The amount of agricultural lime (CaCO_3) and fertilizers used on STK treatment were based from these analyses.

Table 1. Soil pH and amount of lime applied

FARM LOCATION	SOIL pH	AMOUNT OF AGRICULTURAL LIME (CaCO_3)	
		Lime Recommendation (kg/ha) ^a	Actual Amount of Lime Applied (kg/ha) ^b
Egan, Tadian	=6.0	4,942	6,000
Ambasing, Sagada	=6.0	4,942	6,000
Amgayang, Tadian	5.5	4,942	6,000

^a Based on pH 5, because lime recommendation provided in the LaMotte soil test kit is for pH 4, 5, and 6.

^b The equivalent of 4,942 kg/ha is 1.24 kg lime/plot, but this was raised to 1.5 kg/plot (or 6,000 kg/ha) for convenience.

Table 2. Amounts and kinds of fertilizers applied to treatments on farmer's practice and bean fertilizer requirement

TREATMENT	AMOUNT AND KINDS OF FERTILIZERS APPLIED (kg /ha)	
Farmer's Practice	2,000.0	Tripple-14 (14-14-14)
Bean Fertilizer Requirement	65.2	urea (46-0-0)
	556.0	solophos (0-18-0)
	68.0	muriate of potash (0-0-60)

Table 3. Amounts and kinds of fertilizers applied to Treatment 5 (STK) based on the fertilizer recommendations from soil test results (La Motte STK) of the farms

FARM LOCATION	SOIL TEST RESULT, NP ₂ O ₅ K ₂ O (Fertilizer recommendation)	AMOUNT AND KINDS OF FERTILIZERS APPLIED (kg /ha)	
Egan, Tadian	VL – VL – ML, NP ₂ O ₅ K ₂ O (244 – 293 – 171 kg NP ₂ O ₅ K ₂ O/ha)	532	urea (46-0-0)
		1,628	solophos (0-18-0)
		285	muriate of potash (0-0-60)
Ambasing, Sagada	VL – M to ML – MH to M, NP ₂ O ₅ K ₂ O (244 – 195 to 220 – 122 to 146 kg NP ₂ O ₅ K ₂ O/ha)	532	urea (46-0-0)
		1,084 – 1,224	solophos (0-18-0)
		204 – 244	muriate of potash (0-0-60)
Amgayang, Tadian	ML – H – VH, NP ₂ O ₅ K ₂ O (171 – 146 – 73 kg NP ₂ O ₅ K ₂ O/ha)	372	urea (46-0-0)
		812	solophos (0-18-0)
		122	muriate of potash (0-0-60)

Legend: VL – very low ML – medium low H – high
M – medium MH – medium high VH – very high

The need for BNF technology can be assessed with the response of beans to inoculation, which may be either positive or negative. Good nodulation (higher counts of nodules) indicates positive response while poor nodulation indicates otherwise. Response to inoculation was thus assessed by counting the number of nodules in bean root samples.

Good nodulation, however, is not always equated to N₂-fixation and thereby yield, because nodules are either effective or ineffective. Only effective nodules, inhabited by active *Rhizobium*, are capable of N₂-fixation. This means that yield may only be affected significantly by the presence of effective nodules as consequence of N₂-fixation.

Response to inoculation. Beans grown in Egan farm responded positively to inoculation (Table 4). This was demonstrated with higher number of nodules formed (nodulation) in the roots of beans inoculated with a commercial strain of *Rhizobium leguminosarum* bv. *phaseoli* and the highly significant difference obtained among treatment means in the analysis of variance (ANOVA). However, there was a negative response to inoculation from beans both in Amgayang and in Ambasing farms as shown in the non-significant result of the ANOVA among treatment means. Musando and Joshua (2001) also observed lack of response to inoculation in their field trials in Kenya, whereby *Rhizobium* inoculation did not influence pod and seed numbers per plant for the 1998 season and the bean grain field weight for both the 1998 and 1999 seasons. Indeed, even when strains that are more efficient are introduced into the soil, there is no guarantee these strains will compete well with native strains for entry into plant roots (Brick, 2004).

Table 4. Means of the nodulation in beans in the three farms

TREATMENTS	EGAN*	AMGAYANG	AMBASING
T0 – Farmer’s Practice, FP (unlimed), control	0.333 ^b	39.500 ^b	126.000 ^{ab}
T1 – Farmer's Practice, FP (limed)	31.666 ^b	55.000 ^b	89.666 ^{ab}
T2 – BNF	173.000 ^a	108.500 ^{ab}	153.000 ^{ab}
T3 – BNF + FP	51.333 ^b	101.500 ^{ab}	126.333 ^{ab}
T4 – BNF + Bean Fertilizer Requirement	88.666 ^{ab}	158.500 ^a	187.666 ^a
T5 – Soil Test Kit Analysis (STK)	6.333 ^b	87.000 ^{ab}	53.000 ^b
T6 – Bean Fertilizer Requirement	58.000 ^b	57.500 ^b	127.333 ^{ab}

*Means followed by a common letter are significantly different at 5% level (DMRT).

The negative response of beans to inoculation in both Amgayang and Ambasing indicates that indigenous populations of *rhizobium* in the soil can compete with the commercial strain. It also means that it is as infective as the commercial strain. Although, there was no significant difference among the treatment means in Amgayang and Ambasing, nodulation is generally higher in beans that were inoculated. However, analysis of the means with DMRT showed that Treatment 4 (BNF + BFR) differ significantly at 5% level against Treatments 0 and 1 (FP), and Treatment 6 (BFR) in Amgayang and against Treatment 5 (STK) in Ambasing. This implies that the inoculant survived and was able to infect the roots of beans to form nodules. There was no direct evidence though to prove if the commercial strain was indeed the *rhizobium* that caused nodulation, because of the possibility of competition with the indigenous populations of *rhizobium* in the soils of Amgayang and Ambasing.

It is also possible that the existing population of *rhizobium* in the soils of Amgayang and Ambasing was enough and maintained to a sufficient level through yearly or continuous cultivation of beans. This also explains the positive response of beans to inoculation in Egan, whereby kidney beans, peanut, pigeon pea locally known as “cardis”, cowpea (*Vigna unguiculata* ssp. ‘*unguiculata*’), and other legumes are seldom cultivated yearly (personal interview with the farmer cooperator). The distribution of *rhizobium* in soils can vary even within one field and numbers may decline when legumes are not grown (Dart, 1974).

Furthermore, Vincent (1970) stated that legume crops do not always respond positively to inoculation. Lack of response may be because of adequate

natural nodulation, the applied inoculum does not become established (through failure to survive or colonize, or compete with indigenous *rhizobia*), or there are conditions unfavorable for nodule formation and functioning (moisture, temperature, nutrient deficiency, combined N). The second and latter cause, though, is ruled out in this scenario because of higher nodulation in treatments that received inoculation.

Rhizobium strain performance. The inoculant was able to compete with the indigenous populations of *rhizobium* in the soil and was able to survive with the existing soil and climatic condition in the field. *Rhizobium leguminosarum* bv. *phaseoli* likewise did not lose its infectivity as evidenced by higher number of nodules in inoculated beans. Yet, there was no significant difference on the yield of all treatments tested in the three farms.

Analysis of the means through DMRT (Table 5) revealed, though, that the yield of Treatment 5 (STK) in Egan differ significantly at 5% level with that of the other treatments except for Treatments 1 (FP, limed) and 3 (BNF + FP). Likewise in Amgayang, Treatment 1 (FP, limed) and Treatments 3 (BNF) and 4 (BNF + BFR) differ significantly at 5% level (DMRT) but comparable with the other treatments.

The result, thus, implies that the commercial strain of *rhizobium* as well as the indigenous population of *rhizobium* in the three farms was efficient in affecting the yield of beans. Using the commercial strain alone revealed that a comparable yield with the application of inorganic fertilizer could be obtained. Yield, though, is not a good measure of the symbiotic effectiveness and N₂-fixing effectiveness of a *rhizobium*; because, both are based on the total foliage nitrogen content of the legume crop inoculated with the standard strain and the legume crop inoculated with the test strain, and non-fixing crop.

Table 5. Means of the yield (g) of beans in the three farms

TREATMENTS	EGAN*	AMGAYANG	AMBASING	
			First Harvest	Second Harvest
T0 – Farmer’s Practice, FP (unlimed), control	600.000 ^b	545.000 ^{ab}	733.333 ^a	583.333 ^a
T1 – Farmer's Practice, FP (limed)	966.666 ^{ab}	775.000 ^a	516.666 ^a	600.000 ^a
T2 – BNF	433.333 ^b	350.000 ^b	733.333 ^a	416.666 ^a
T3 – BNF + FP	1016.666 ^{ab}	525.000 ^{ab}	678.333 ^a	533.333 ^a
T4 – BNF + Bean Fertilizer Requirement	700.000 ^b	400.000 ^b	616.666 ^a	450.000 ^a
T5 – Soil Test Kit Analysis (STK)	1400.000 ^a	500.000 ^{ab}	783.333 ^a	466.666 ^a
T6 – Bean Fertilizer Requirement	400.000 ^b	555.000 ^{ab}	550.000 ^a	400.000 ^a

*Means followed by a common letter are significantly different at 5% level (DMRT).

BNF technology performance. Results revealed that the BNF technology is comparable with other agricultural practices including the farmer’s practice (Table 5). However, in terms of fertilization cost, the use of inoculant for bean cultivation is generally cheaper than the farmer's practice (Table 6). It is environment-friendly because soil and water pollution or related problems (e.g, increased soil acidity and nitrate enrichment of ground water) caused by continued application of nitrogen fertilizers can be minimized if not prevented with the use of BNF technology.

Table 6. Cost comparison of fertilization among treatments

TREATMENTS	LIME & FERTILIZER INPUTS PER TREATMENT	UNIT PRICE OF LIME OR FERTILIZER (PESO)	AMOUNT OF LIME OR FERTILIZER USED (kg) ^b	COST OF LIME OR FERTILIZER USED (PESO) ^b	TOTAL COST OF LIME & FERTILIZER USED (PESO)	
					Per Treatment ^b	Per Hectare
T0 – Farmer’s Practice, FP (unlimed), control	complete fertilizer (T-14)	9.50/kg	4.00	38.00	38.00	19,000.00
T1 – Farmer's Practice, FP (limed)	complete fertilizer (T-14)	9.50/kg	4.00	38.00	62.00	31,000.00
	lime material	100.00/bag (50kg)	12.00	24.00		
T2 – BNF	legumin	7.50/packet ^a		7.50	31.50	15,750.00
	lime material	100.00/bag (50kg)	12.00	24.00		

Table 6. Continued . . .

TREATMENTS	LIME & FERTILIZER INPUTS PER TREATMENT	UNIT PRICE OF LIME OR FERTILIZER (PESO)	AMOUNT OF LIME OR FERTILIZER USED (kg) ^b	COST OF LIME OR FERTILIZER USED (PESO) ^b	TOTAL COST OF LIME & FERTILIZER USED (PESO)	
					Per Treatment	Per Hectare
T3 – BNF + FP	legumin	7.50/packet		7.50	69.50	34,750.00
	lime material	100.00/bag (50kg)	12.00	24.00		
	complete fertilizer (T-14)	9.50/kg	4.00	38.00		
T4 – BNF + Bean Fertilizer Requirement	legumin	7.50/packet		7.50	40.43	20,216.80
	lime material	100.00/bag (50kg)	12.00	24.00		
	urea (46-0-0)	9.00/kg	0.13	1.17		
	solophos (0-18-0)	300.00/bag (50kg)	1.11	6.67		
	muriate of potash (0-0-60)	8.00/kg	0.14	1.09		

Table 6. Continued . . .

TREATMENTS	LIME & FERTILIZER INPUTS PER TREATMENT	UNIT PRICE OF LIME OR FERTILIZER (PESO)	AMOUNT OF LIME OR FERTILIZER USED (kg) ^b	COST OF LIME OR FERTILIZER USED (PESO) ^b	TOTAL COST OF LIME & FERTILIZER USED (PESO)	
					Per Treatment ^b	Per Hectare
T5 – Soil Test Kit Analysis (STK)	urea (46-0-0)	9.00/kg	0.98	8.86	27.29	13,643.36
	solophos (0-18-0)	300.00/bag (50kg)	2.48	14.85		
	muriate of potash (0-0-60)	8.00/kg	0.45	3.58		
T6 – Bean Fertilizer Requirement	urea (46-0-0)	9.00/kg	0.13	1.17	8.93	4,466.80
	solophos (0-18-0)	300.00/bag (50kg)	1.11	6.67		
	muriate of potash (0-0-60)	8.00/kg	0.14	1.09		

^a The inoculant can be used for 20 kg seed at a rate of 1 tbsp. inoculant/kg seed.

^b Total per treatment for the three farms (20 m²/treatment = 8 plots/treatment)

Furthermore, the inoculant can be utilized for the succeeding cropping of the same legume crop because of their ability to survive in the soil and compete with the existing population of *rhizobium*, which are either less infective or effective, provided the time interval is not a year or more.

Statistical analysis showed that there was no significant difference on the treatment means of the yield of the agricultural practices tested (Table 5). On the other hand, higher yields observed on some treatments could be the influence of the application of inorganic fertilizers, liming and soil or all of the three factors.

The BNF technology (treatment 2) generally has lower yield than the farmer's practice or treatment 0 (Table 5), but the former has higher counts of nodules (Table 4). Even the combination of BNF technology with other practices had lower yields than the farmer's practice, limed or unlimed.

Nodulation, however, in the farmer's practice either limed or unlimed is generally lower than that of the other practices. These results agree with Brick's statement (2004) that attempts to supplement the legume nitrogen supply (from the atmosphere through symbiotic nitrogen fixation) by fertilization usually are counterproductive, because plants tend to stop nitrogen fixation when soil nitrogen is high.

Results also indicated that higher yields are obtained when nodule counts are lower (Figure 1).

Simple regression and correlation analyses made between nodulation and bean yield on the three farms showed a negative linear correlation (Figures 2, 3 and 4); unlike in the study of Elias, et al. (n. d.) whereby chickpea yield was found to be positively aligned with nodulation score. In like manner, low but significant correlation was also found between extent of nodulation and seed yield of soybeans (Brockwell, et al.; 2006), and positive correlation between nodulation and peanut yield despite the few inconsistencies observed on nodulation among some plants at each grid point in the experiment (Trostle, 2002).

It can be inferred, thus, that other factors such as fertilization (treatment effects) and ineffective nitrogen fixation to name a few could have influenced the yield. Further studies are therefore suggested to investigate the relation between nodulation and yield under a specified cropping management.

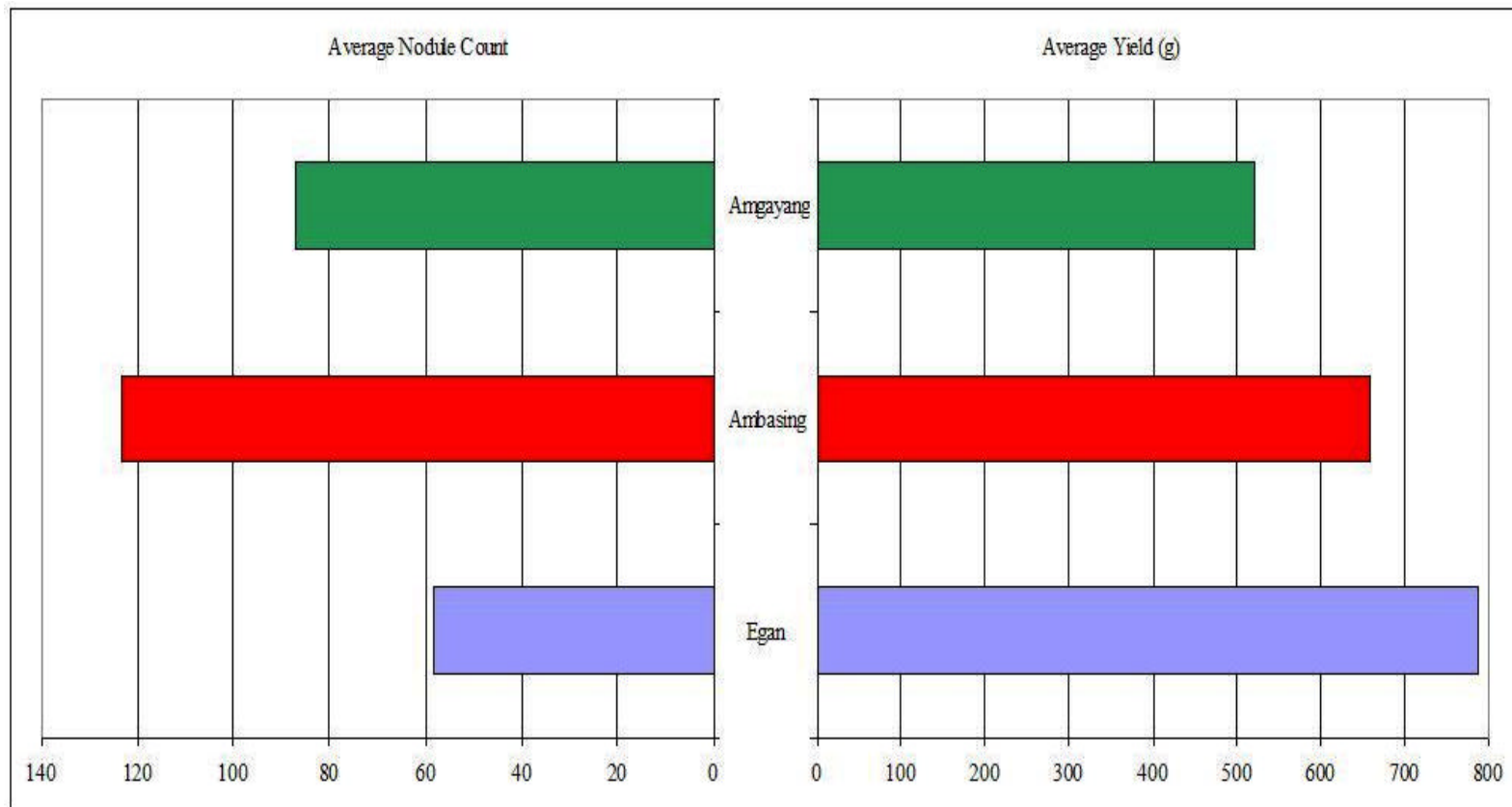


Figure 1. Average nodule count and yield (grams) of beans

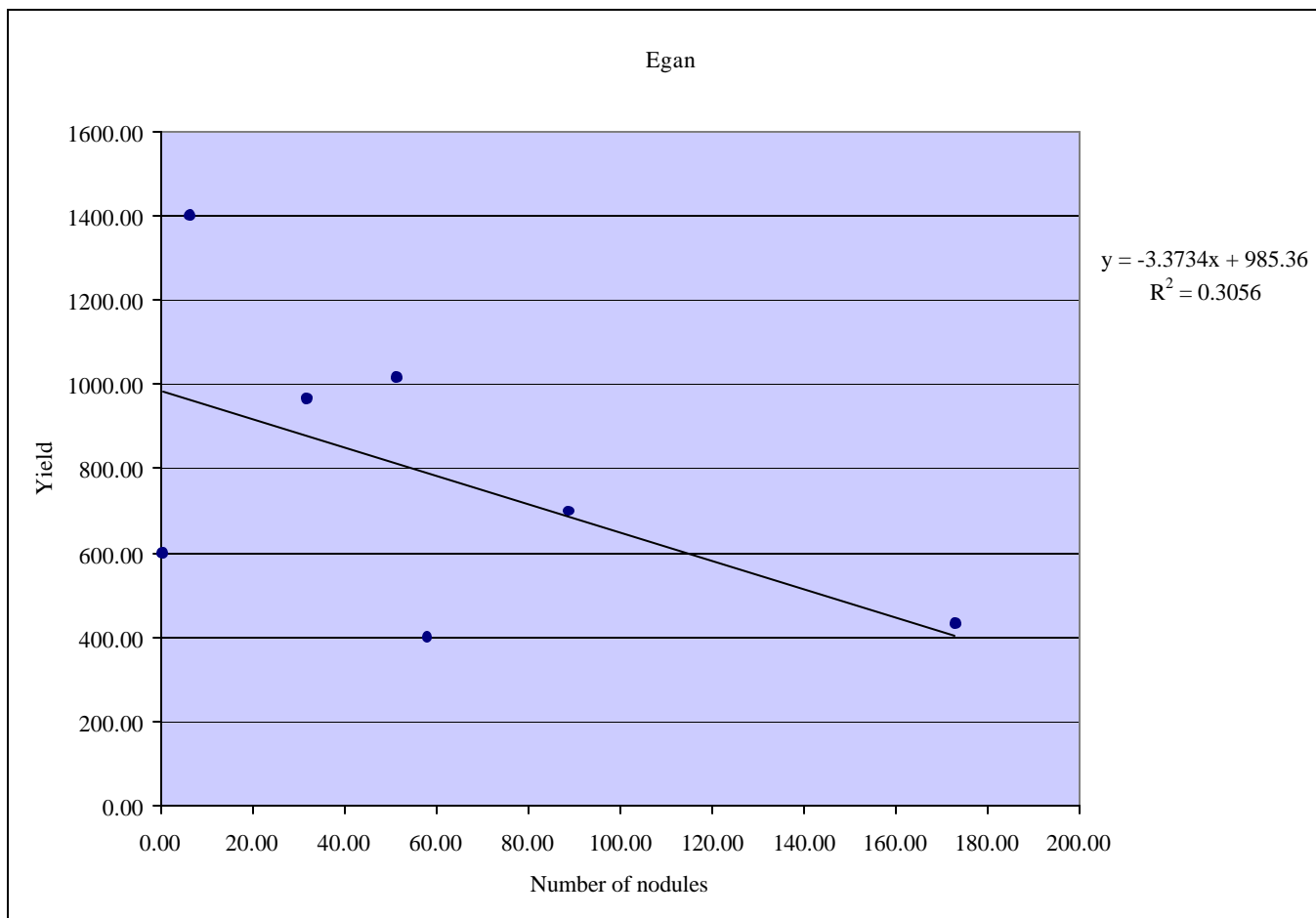


Figure 2. Regression and correlation analysis between nodulation and yield of beans in Egan farm

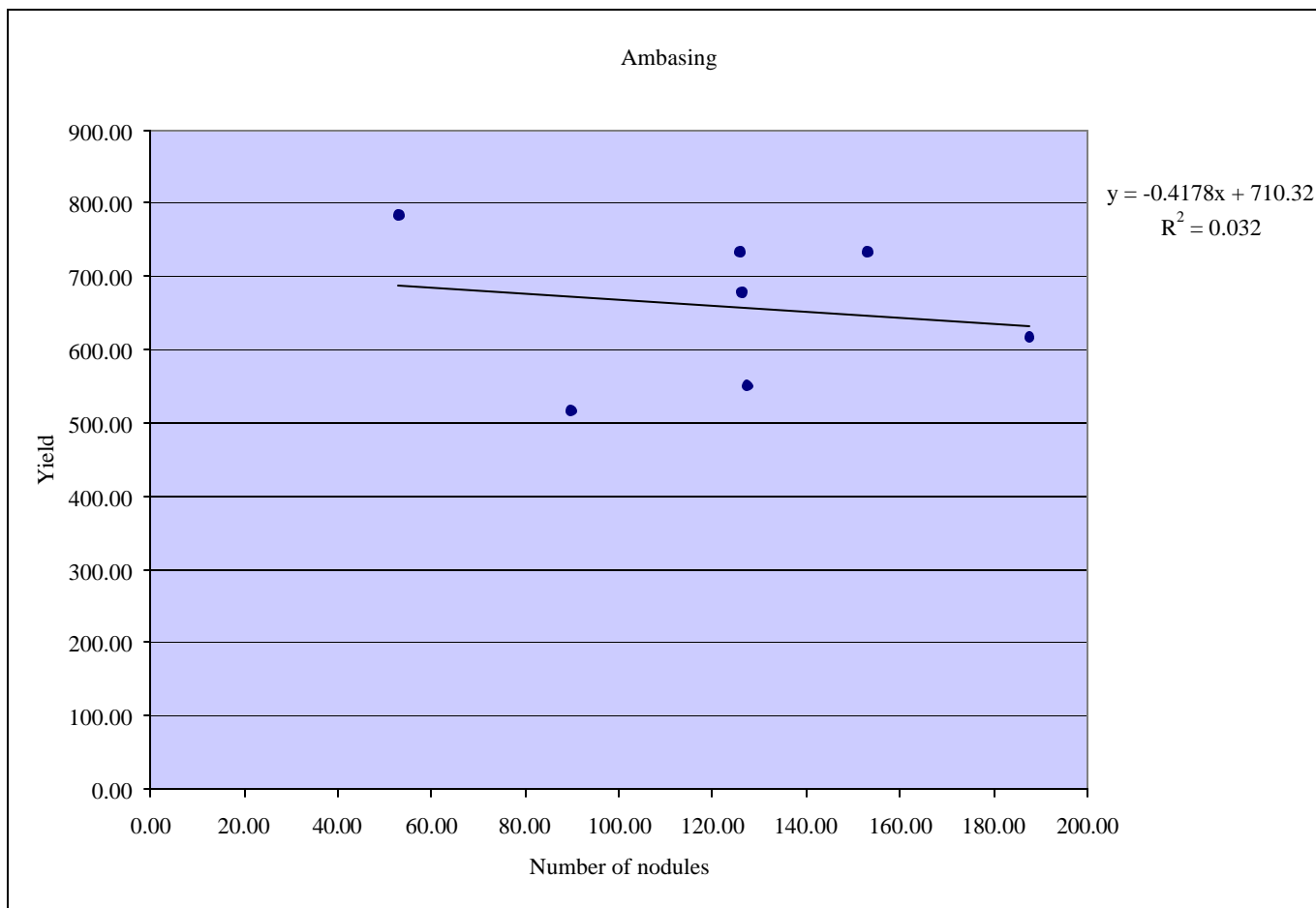


Figure 3. Regression and correlation analysis between nodulation and yield of beans in Ambasing farm

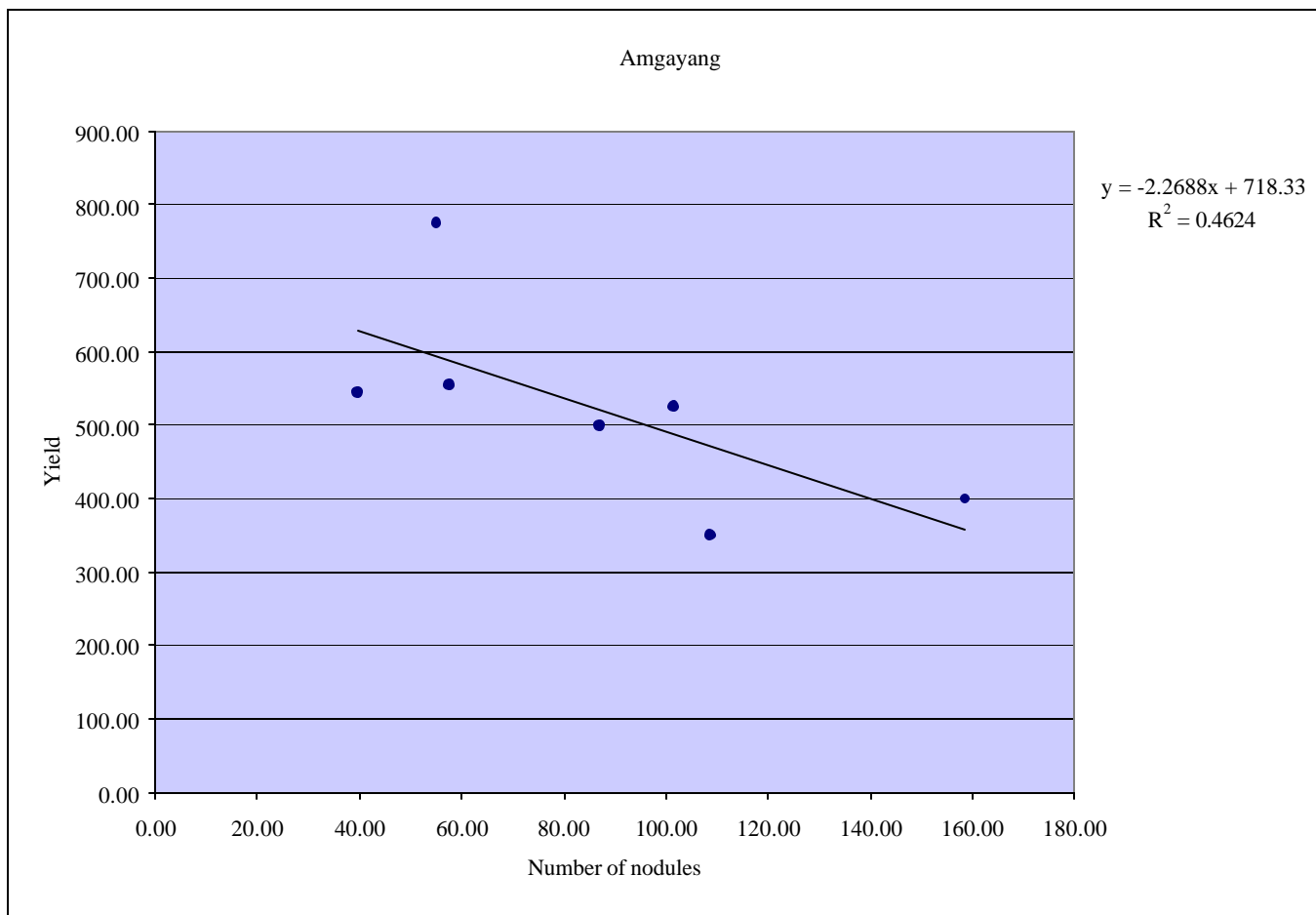


Figure 4. Regression and correlation analysis between nodulation and yield of beans in Amgayang farm

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

This study was done, specifically, to (1) assess the need for bean inoculation (BNF technology in beans) in three selected farms, and (2) compare BNF technology with other agricultural practices in the cultivation of beans (*Phaseolus vulgaris*) as to their effect to yield and nodulation.

Three farmer cooperators were identified and had their farms utilized for the trials. Three months prior to sowing, the farms were amended with agricultural lime (CaCO₃). Liming was based on soil pH analyses of the three farms using the La Motte soil test kit. The farms had a pH ranging from 5.5 to =6.0. The actual amount of lime applied per plot was 1.5 kg. Only the treatment plots with BNF technology and treatment 1 plot were limed.

Black pole bean (*Phaseolus vulgaris*) of 'Alno' cultivar was used as the host plant with the commercial inoculant, Legumin, containing *Rhizobium leguminosarum* bv. *phaseoli* strain as inoculum. Fertilizers given to other treatments without BNF were applied as sidedress.

Results revealed that only the beans cultivated at Egan farm responded positively to inoculation, most likely due to sparse population of indigenous *Rhizobium* in the soil. This result suggests that the inoculum can compete with indigenous *Rhizobium* population in the soil when population is few. Inoculated beans had higher nodulation counts than uninoculated beans but, generally, had lower yields. Statistical analyses, however, showed that yields between the inoculated and uninoculated treatments were not significant.

From these results, it can be inferred that the inoculum is infective given the higher nodulation counts in inoculated treatments, especially in Egan farm. Yet, correlation between nodulation and yield was negative, albeit linear. The yield, thus, might have been influenced not only by nitrogen fixation but also of other factors like fertilization.

Conclusions

- 1.) In Egan, Tadian, there is a need for inoculation; in Amgayang, Tadian, and Ambasing, Sagada, inoculation is not needed. If the cultivation of beans, though, is stopped for a long time, inoculation may be necessary.

- 2.) The commercial strain *Rhizobium leguminosarum* bv. *phaseoli* obtained from BIOTECH, UPLB, may be used as source of inoculant in the selected farms—Tadian and Sagada, because of its ability to adapt with the existing climatic condition, survive, and compete with the indigenous population of *rhizobium*.
- 3.) BNF technology is still of advantage in the cultivation of beans compared to the farmer's practice due to cheaper cost of fertilization, and against other agricultural practices including the farmer's practice because it is environment-friendly.

Recommendations

- 1.) More farm trials should be made to assess the need for inoculation and extend to farmers the innovation in farming in light of organic farming and sustainable agriculture. Although, assessment indicated that there is no need for inoculation, there is no danger in applying inoculant. This is even encouraged to ensure sufficient number of *rhizobium* in the soil during sowing.
- 2.) Since BNF technology showed a comparable yield to that of the farmer's practice, it should be disseminated to farmers as a way to encourage organic farming and decrease soil-related problems or pollution because of the use of inorganic nitrogen fertilizers. Besides, fertilization cost of the technology is lesser compared to farmer's practice.

REFERENCES

- ADJEI, M. B.; K. H. QUESENBERRY, and C. G. CHAMBLISS. 2002. Nitrogen Fixation and Inoculation of Forage Legumes. <http://edis.ifas.ufl.edu/AG152>
- AGRI-FACTS. 2002. Pea nutrient requirements in Alberta. Practical Information for Alberta's Agriculture Industry. Agdex 142/532-2. April 2002. [http://www1.agric.gov.ab.ca/\\$department/deptdocs.nsf/all/agdex4650/\\$file/142_532-2.pdf?OpenElement](http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex4650/$file/142_532-2.pdf?OpenElement)
- ALEXANDER, M. 1977. Introduction to soil microbiology. John Wiley & Sons, Inc. 2nd ed.

- BAUTISTA, O. K. and R. C. MABESA. 1986. Vegetable production. Integrated Food and Agricultural Research Training and Extension Program and the National Food and Agricultural Council Department of Agriculture. 3rd ed.
- BEUERLEIN, J. 2001. Effect of soybean inoculation on grain yield in Ohio in 2001. The Ohio State University, Extension/Research Information. <http://www.oardc.ohio-state.edu/soy2001/inoculationtrials.htm>
- BIOMAT NET. 2003. Ahipa: exploring the potential of a sustainable crop as an alternative non-food source. <http://www.biomatnet.org/secure/Fair/R4297.htm>
- BIRO, B. ; I. VOROS; K. KOVESPECHY; and J. SZEGI. 1993. Symbiont effect of *Rhizobium* bacteria and vesicular arbuscular mycorrhizal fungi on *Pisum sativum* in recultivated mine spoils. http://www.osti.gov/energycitations/product.biblio.jsp?osti_id=7054648
- BRADY, N. C. and R. R. WEIL. 1996. The nature and properties of soils. Prentice-Hall, Inc. A Simon and Schuster Co. Upper Saddle River, N.J. 11th ed.
- BRICK, M. A. 2004. Legume Seed Inoculants. <http://www.ext.colostate.edu/pubs/crops/00305.html>
- BROCKWELL, J.; R. R. GAULT; D. L. CHASE; G. L. TURNER; and F. J. BERGERSEN. 2006. Establishment and expression of soybean symbiosis in a soil previously free of *Rhizobium japonicum*. <http://www.publish.csiro.au/paper/AR9930137.htm>
- BRYAN, A. H.; C. A. BRYAN; and C. G. BRYAN. 1962. Bacteriology: principles and practice. (Philippine Copyright, 1970 by Barnes & Noble, Inc.) Barnes & Noble, Inc. 6th ed.
- CIDICCO. 2003. Report on preliminary results obtained on the utilization of velvetbeans (*Mucuna* sp.) at Montelibano Agroindustries, Choluteca, Honduras. CIDICCO, Cover Crops International Clearing House. <http://cidicco.hn/newcidiccoenglish/inf13.htm>
- COSICO, W. C. and P. A. POSELION. 1988. Transfer of microbial inoculant technologies from BIOTECH to the farm. BIOTECH Research Abstracts. National Institute of Biotechnology and Applied Microbiology (BIOTECH), UPLB, College, Laguna, Phil.
- CSIDC. 2006. Fertility management for dry bean production under irrigation. http://www.agr.gc.ca/pfra/csfdc/beanfert_e.htm

- DANSO, S. 1998. Nitrogen fixation through *Rhizobium* inoculation (GHA/5/024). Soils Newsletter. A Publication of the Soil and Water Management and Crop Nutrition Sub-Programme of the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and FAO/IAEA Agriculture and Biotechnology Laboratory, Seibersdorf International Atomic Energy Agency Vienna, Austria. [http://www.Fertilizer Suggestions For Soybeans, G87-859-A \(Revised August 2003\)-pub.iaea.org/MTCD/publications/PDF/Newsletters%5CSW-NL-21-2.pdf](http://www.Fertilizer Suggestions For Soybeans, G87-859-A (Revised August 2003)-pub.iaea.org/MTCD/publications/PDF/Newsletters%5CSW-NL-21-2.pdf)
- DART, P. J. 1974. Biological nitrogen fixation. Consultative Group on International Agricultural Research. Technical Advisory Committee. Rothamsted Experimental Station.
- ELIAS, N. V; R. J. MARTIN; J. F. HOLLAND; P. HAYMAN; M. ROBERTSON; J. GORDON; and S. BELFIELD. n. d. Broadleaf crops for diversification of dryland cropping in the northern wheat-belt of Eastern Australia. http://www.cropscience.org.au/icsc2004/poster/2/1/1/1276_elias_nv.htm?print=1
- FERGUSON, R. B.; C. A. SHAPIRO; A. R. DOBERMANN; and C. S. WORTMANN. 2003. Fertilizer recommendations for soybeans. Nebraska Cooperative Extension G87-859-A. University of Nebraska-Lincoln. <http://ianrpubs.unl.edu/FieldCrops/g859.htm>
- FINCK, A. 1982. Fertilizers and fertilization: introduction and practical guide to crop fertilization. Verlag Chemie GmbH, Weinheim.
- GARCIA, M. W. and T. S. SAN JOSE. 1983. International seminar on productivity of soil ecosystems. NRI. Tokyo University of Agriculture.
- GRAIN LEGUMES. n. d. Nitrogen fixation and yield of grain legume in saline Mediterranean zones (FYSAME). http://www.grainlegumes.com/ulf/aep/euro_project/fichier_13.DOC
- HARDMAN, H. 2005. Plant hemoglobins: Oxygen handlers critical for nitrogen fixation. http://www.eurekalert.org/pub_releases/2005-03/cppho032305.php
- HARDARSON, G. and C. ATKINS. 2001. Optimising biological nitrogen fixation by legumes in farming systems. Technical Expert Meeting on Increasing the Use of Biological Nitrogen Fixation (BNF) in Agriculture. FAO, Rome. <http://www.iaea.org/programmes/nafa/d1/crp/bnf-march2001.pdf#search='bean%20nitrogen%20fixation%20farm%20trials'>

- IIHR, n. d. Biofertilizer laboratory. http://www.iihr.res.in/BIO_TECH_biofer_tili.htm
- INFOPEdia. 1995. Nitrogen fixation. Softkey International Inc. USA. INP3AE—F1.
- KEETON, W. T. and C. H. MCFADDEN. 1983. Elements of biological science. W. W. Norton and Co., Inc. 3rd ed.
- KUMAZAWA, K. and T. OHYAMA. 1983. International seminar on productivity of soil ecosystems. NRI. Tokyo University of Agriculture.
- LAMOTTE COMPANY. 1995. Garden guide manual for LaMotte soil chemistry test equipment. LaMotte Company, USA. Code 500.
- MARUYAMA, Y. and H. HORITA. 1983. International seminar on productivity of soil ecosystems. NRI. Tokyo University of Agriculture.
- MENDOZA, T. C.; E. B. AROMIN; E. R. CELESTINO; and C. J. ANDAM. 1993. The Philippine agriculturist. Journal of the College of Agriculture and the Central Experiment Station. UPLB. 76(2).
- MERESTELA, T. M.; D. M. APLATEN; D. Y. FOMEG-AS; L. T. KITONGAN; and E. B. LAYAO. 1996. Abundance of *R. leguminosarum* bv. *viceae* and *phaseoli* in some soils of Benguet and Mountain Province. BSU Res. Journal. Benguet State University, La Trinidad, Benguet.
- MUSANDO, A. A. O. and O. O. JOSHUA. 2001. Response of common bean to *Rhizobium* inoculation and fertilizers. <http://www.bioline.org.br/request?ft01032>
- PATERNO, E. S. 1984. Evaluation of the *Rhizobium* requirement of food legumes over a wide range of environmental conditions. BIOTECH Research Abstracts. National Institute of Biotechnology and Applied Microbiology (BIOTECH), UPLB, College, Laguna, Phil.
- _____. 1984. Isolation and identification of rhizobial strains tolerant to adverse conditions. BIOTECH Research Abstracts. National Institute of Biotechnology and Applied Microbiology (BIOTECH), UPLB, College, Laguna, Phil.
- _____; M. L. Q. SISON; E. S. GARCIA; F. G. TORRES; and A. I. SUTARE. 1995. Population of *Rhizobia* and mungbean response to frequency of inoculation in a rice-based cropping system. The Philippine Journal of Biotechnology. vol. 6, no. 1.

- _____; F. G. TORRES; M. L. Q. SISON; and E. S. GARCIA. 1985. Utilization of *rhizobium* technology in food legume production. BIOTECH Research Abstracts. National Institute of Biotechnology and Applied Microbiology (BIOTECH), UPLB, College, Laguna, Phil.
- _____. 1982. Effects of the different methods of inoculation on the yield and nodulation of mungbean (*Vigna radiate* (L) Wilczek). BIOTECH Research Abstracts. National Institute of Biotechnology and Applied Microbiology (BIOTECH), UPLB, College, Laguna, Phil.
- _____. 1982. Effects of the different methods of inoculation on the yield and nodulation of peanut (*Arachis hypogaeae* L.). BIOTECH Research Abstracts. National Institute of Biotechnology and Applied Microbiology (BIOTECH), UPLB, College, Laguna, Phil.
- POSTGATE, F. R. S. 1982. The fundamentals of nitrogen fixation. Cambridge University Press.
- RAVEN, P. H. and G. B. JOHNSON. 1989. Biology. Times Mirror/Mosby College Publishing. 2nd ed.
- ROMERO, E. M. 2001. Role of nitrogen fixation in common bean based cropping systems in Latin America: an overview of bean *Rhizobium* symbiosis. Technical Expert Meeting on Increasing the Use of Biological Nitrogen Fixation (BNF) in Agriculture. FAO, Rome. <http://www.iaea.org/programmes/nafa/d1/crp/bnf-march2001.pdf#search='bean%20nitrogen%20fixation%20farm%20trials'>
- SALISBURY, F. B. and C. W. ROSS. 1985. Plant physiology. Wadsworth, Inc. 3rd ed.
- SANCHEZ, P. A. 1976. Properties and management of soils in the Philippines. New York; John Wiley & Sons, Inc.
- SANTALLA, M.; J. M. AMURRIO; and A. M. DE RON. 2001. Symbiotic interactions between *Rhizobium leguminosarum* strains and elite cultivars of *Pisum sativum* L.. Journal of Agronomy and Crop Science. 187 (1): 59-68. <http://www.blackwell-synergy.com/doi/abs/10.1046/j.1439-037X.2001.00502.x>
- SINGER, M. J. and D. N. MUNNS. 1987. Soils: an introduction. Macmillan Publishing Co., N.Y.

- SISON, M. L. and E. S. PATERNO. 1995. Population dynamics of *Bradyrhizobium japonicum* (Kirchner) Jordan in rice-soybean rotation. The Philippine Journal of Biotechnology. vol. 6, no. 1.
- SLATTERY, J. and D. PEARCE. n. d. The impact of background *rhizobial* populations on inoculation response. [http://www.aciar.gov.au/web.nsf/att/JFRN-6BN922/\\$file/pr109echapter05.pdf](http://www.aciar.gov.au/web.nsf/att/JFRN-6BN922/$file/pr109echapter05.pdf)
- SLATTERY, J.; D. PEARCE; M. RAYNES; D. CARPENTER; G. DEAN; and M. MATERNE. n. d. Variation in yield of faba bean across Southern Australia following rhizobial inoculation. <http://www.regional.org.au/asa/2003/c/4/slattery.htm?print=1>
- STARR, C. and R. JAGGART. 1989. Biology: the unity and diversity of life. Wadsworth, Inc. 5th ed.
- THE SOYBEAN COMMITTEE. 1975. The Philippine recommends for soybean 1976. PCAR.
- TORTORA, G. J.; B. R. FUNKE; and C. L. CASE. 1986. Microbiology: an introduction. The Benjamin/Cummings Publishing Co., Inc. 2nd ed.
- _____. 1989. Microbiology: an introduction. The Benjamin/Cummings Publishing Co., Inc. 3rd ed.
- TRINIDAD, L. G. and T. YOSHIDA. 1989. Optimization of growth parameters for scale-up production of some N₂-fixing organisms. Annual Reports of ICBIOTECH. International Center of Cooperative Research in Biotechnology, Japan, Fac. of Eng'g, Osaka Univ., Osaka, Japan. 12
- TROSTLE, C. 2002. Precision agriculture initiative for Texas high plains: 2002 Annual Comprehensive Report.
- VINCENT, J. M. 1970. A manual for the practical study of root nodule bacteria. IBP Handbook No. 15. Blackwell Scientific Publications, Oxford and Edinburgh.
- VITOSH, M. L. 1997. Soybean inoculation in Michigan. Soybean Facts Winter. Michigan State University Extension. <http://web1.msue.msu.edu/imp/mods1/fact9708.html>
- VOLK, W. A. and M. F. WHEELER. 1988. Basic microbiology. Harper and Row, Publishers, Inc. 6th ed.
- WALKER, N. 1975. Soil microbiology. Butterworth & Co (Publishers) Ltd.

ZAHARAN, H. H. 1999. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and Molecular Biology Reviews. Dec. 1999. 63(4): p. 968-989. <http://www.pubmedcentral.nih.gov/picrender.fcgi?artid=98982&blobtype=pdf>