MULTI-FARM TRIAL OF BIOLOGICAL NITROGEN FIXATION (BNF) IN BEANS (*Phaseolus vulgaris*) AT MOUNTAIN PROVINCE[1]

by

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KEY WORDS: lime, inoculum, inoculant, rhizobium, biological nitrogen fixation, dinitrogen fixation, bean's fertilizer recommendation, soil test kit, farmer's practice, nodulation

ABSTRACT

The BNF technology was extended to farmer cooperators in Egan and Amgayang, Tadian, and Ambasing, Sagada—all in Mountain Province—through demo farms with the aims of assessing the need for inoculation in their farms, determining the performance of the standard strain inoculant, and comparing the effects on nodulation and yield of beans of the assigned treatments especially the inoculant *Rhizobium leguminosarum* bv. *phaseoli* (CIAT) vis-à-vis the farmer's practice. Inoculated beans showed higher nodulation compared to the other uninoculated beans or treatments. However, only in Egan, Tadian did the inoculated beans showed a positive response to inoculation based on the ANOVA, most likely due to sparse population of local rhizobium in the soil. Yield of inoculated beans was generally lower than other practices including the farmer's practice, but were comparable based on the ANOVA or there was no significant difference found among treatment means on yield. Nodulation of beans in farmer's practice either limed or unlimed is generally lower than the other practices tested. Also, all treatments with BNF technology including the BNF technology alone as treatment are generally of higher nodulation than the rest of the treatments tested. But, all treatments with BNF technology or inoculated beans generally had lower yields than farmer's practice either limed or unlimed.

INTRODUCTION

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, local 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.

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Beans are commonly cultivated in some places of Mountain Province. Some bean growers usually produce for market while others cultivate them both for market and home consumption.

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 native or local 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.

Inoculation is beneficial in two ways. First, it improves nodulation and dinitrogen fixation; second, it may increase rhizobium population in the soil. Higher number or population of rhizobium increases nodulation rate; therefore may also increase N_2-fixation rate. Generally, legume yields are increased through N_2-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 local rhizobia), or there are conditions unfavorable for nodule formation and functioning (moisture, temperature, nutrient deficiency, combined N) (Vincent, 1970).

Moreover, the native rhizobium or applied inoculum (*Rhizobium* strains) may have poor N_2-fixing effectiveness and symbiotic effectiveness. Field trials assess the effectivity or infectivity of rhizobium and the need for inoculation.

The technology on legume inoculation or leguminous symbiotic/biological nitrogen fixation (BNF) is not yet widely disseminated to farmers. Almost all farmers in the Cordillera are not 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 dinitrogen 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. Dinitrogen 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 local rhizobia due to variable soil environment conditions. Hence, farmers may need to inoculate every time they grow legumes especially when population of native 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 local rhizobium. The field trial will also serve as demo farms to farmers on the application of BNF soil technology in beans. In addition, more location trials on legume inoculation are needed due to variable soil environment conditions, to transfer the technology to farmers, and improve farmer's vegetable and grain legume production.

The study, thus, have the following general objectives (1) To extend BNF technology in beans to farmers in some places of Mountain Province through demo farms, and (2) To determine the performance of BNF technology in beans in some places of Mountain Province through location trials. Specifically, though, the objectives were (1) To assess the need for bean inoculation (BNF technology in beans) in selected places of Mountain Province, (2) To determine and compare the performance of the standard strain (CIAT) of *Rhizobium leguminosarum* biovar *phaseoli* with native or local populations of *Rhizobium leguminosarum* biovar *phaseoli* in terms of nodulation and yield of beans in selected places of Mountain Province, and (3) To compare BNF technology with other agricultural practices in the cultivation of beans (Phaseolus vulgaris) as to their effects on yield and nodulation.

METHODOLOGY

Problems. The following were the problems this research aimed to answer; (1) Is there a need of rhizobium inoculation for beans in some places of Mountain Province?, (2) How would be the performance of the standard strain of rhizobium (CIAT strain) compared to inherent/native soil rhizobium in affecting the nodulation and yield of beans in some locals of Mountain Province?, and (3) Will the effects of BNF technology on nodulation and yield of beans be comparable or better than other agricultural practices like farmer practice?

Methods. Farmer cooperators, cultivating beans, were identified from selected places in Mountain Province. Field trials and or demo farms were done to showcase the biological nitrogen fixation (BNF) technology to farmers. Farmer cooperators helped maintain the demo farms together with the researcher or extensionist. Field trials in beans were made in Ambasing, Sagada (Plate 1); Egan, Tadian (Plate 2); and Amgayang, Tadian (Plate 3). A standard microbial inoculant (*Rhizobium*, CIAT) was obtained from BIOTECH, UPLB, Los Banos, Laguna.

Plate 1. Demo farm in Ambasing, Sagada, Mountain Province

Plate 2. Demo farm in Egan, Tadian, Mountain Province

Plate 3. Demo farm in Amgayang, Tadian, Mountain Province

The primary basis of the performance of the technology was determined from the responses of the crops to inoculation—e.g., nodulation and yield. The crop's response was compared with that of the other treatments. Only the treatments with BNF and T1 were limed. The amount of liming material used (agricultural lime, $CaCO_3$) was determined with the La Motte soil test kit. Liming was done 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 variety. Inoculation was done following the instruction given on the label of the inoculant.

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

The farmer's practice is the application of an estimated one-tablespoon T-14 inorganic fertilizer per hill (personal interview and communication with the farmer cooperators), while the bean fertilizer requirement is simply the recommended rate for bean, which is 30-100-40 kg $NP_2O_5K_2O$/ha (Bautista and Mabesa, 1986). Treatment 5 is based on La Motte soil test kit analysis; thus, the amount of fertilizer material might vary for each farm. Fertilizer materials used for the STK treatment are urea (46-0-0), solophos (0-18-0), and muriate of potash (0-0-60).

Randomized blocking 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 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. The dimensions of the plots used were approximately 0.5 m X 5.0 m or close to this in each field. One value was missing on the data of yield for Egan, hence the use of analysis of covariance (ANOCOVA) for estimating missing data in a randomized complete block design. The analysis of variance (ANOVA) was used for the rest of the data.

Nodulation was determined on the onset of flowering from the average values of five randomly selected samples in each treatment for every replication. Randomly selected samples were carefully uprooted and the nodules counted and recorded as index for nodulation. Yield was likewise recorded during the first harvest taken from the average yield of fifteen randomly selected samples in each treatment for every replication. Due to unavoidable circumstance, succeeding yields and other data (i.e., foliage weight and %N) were not gathered. Nevertheless, this will not adversely affect the outcome of the study in terms of yield interpretation as this was based equally the same on the first harvest. Albeit, the overall yield of beans might better reflect the effects of each treatment on yield.

RESULTS AND DISCUSSION

Bean response to inoculation. Beans grown in Egan demo farm responded positively with inoculation. This was demonstrated with higher number of nodules formed (nodulation) in the roots of beans inoculated with a standard strain of *Rhizobium leguminosarum* bv. *phaseoli* (CIAT) and the highly significant difference obtained among treatment means in the analysis of variance (ANOVA) (Table 1). However, there was a negative response to inoculation from beans cultivated both in Amgayang and Ambasing demo farms as shown in the non significant result of the ANOVA among treatment means (Table 2 and 3, respectively).

Table 1. Analysis of variance (ANOVA) table of bean nodulation in Egan, Tadian demo farm

S.V.	d.f.	SS	MS	F-value	F-tabular	
					0.05	0.01
Block	2	5648.38	2824.19			
Treatment	6	62689.90	10448.32	5.92 **	3	4.82
Error	12	21186.95	1765.58			
Total	20	89525.24				

ns not significant ** highly significant

c.v. = 71.856 std. error of the treatment mean = 24.259

Table 2. Analysis of variance (ANOVA) table of bean nodulation in Amgayang, Tadian demo farm

S.V.	d.f.	SS	MS	F-value	F-tabular	
					0.05	**0.01**
Block	1	10920.07	10920.07			
Treatment	6	19869.86	3311.64	2.63 **ns**	4.38	8.47
Error	6	7562.43	1260.40			
Total	13	38352.36				

ns not significant * significant

c.v. = 40.907 std. error of the treatment mean = 20.497

Table 3. Analysis of variance (ANOVA) table of bean nodulation in Ambasing, Sagada demo farm

S.V.	d.f.	SS	MS	F-value	F-tabular	
					0.05	**0.01**
Block	2	54312.00	27156.00			
Treatment	6	33393.62	5565.60	1.42 **ns**	3	4.82
Error	12	46894.67	3907.89			
Total	20	134600.29				

ns not significant ** highly significant

c.v. = 50.705 std. error of the treatment mean = 36.091

The significant difference and highly significant difference observed among the blocks or replications (Table 2 and Table 3, respectively) may be accounted to limitations on blocking because of the dimension and or size, and orientation of the demo farms as mentioned earlier.

The negative response of beans to inoculation both in Amgayang and Ambasing indicates that local populations of rhizobium in the soil can compete with the standard strain. It also means that it is as infective as the standard strain. Although, there was no significant difference among the treatment means in Amgayang and Ambasing, nodulation is generally higher in beans that were inoculated (Table 4). 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 standard strain was in fact the rhizobium that caused nodulation, because of the possibility of competition with the local or native populations of rhizobium in the soils of Amgayang and Ambasing.

Table 4. Means of the nodulation in beans in the three demo farm

Treatments	Egan	Amgayang	Ambasing
T0 – farmer's practice, FP (unlimed), control	0.333	39.500	126.000
T1 – farmers'practice, FP (limed)	31.666	55.000	89.666
T2 – BNF	173.000	108.500	153.000
T3 – BNF + FP	51.333	101.500	126.333
T4 – BNF + bean fertilizer requirement	88.666	158.500	187.666
T5 – soil test kit analysis (STK)	6.333	87.000	53.000
T6 – bean fertilizer requirement	58.000	57.500	127.333

It is also possible that the already existing populations of rhizobium in the soils of Amgayang and Ambasing were enough and maintained to a sufficient level through yearly or continuous cultivation of beans (personal communication with the farmer cooperators). 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 local 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 (Table 4).

<u>Rhizobium strain performance</u>. The inoculant was able to compete with the local 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* (CIAT) likewise did not lose its infectivity as evidenced by higher number of nodules in inoculated beans (Table 4). Yet, there was no significant difference on the yield of all treatments tested in the three farms (Table 5, Table 6, and Table 7).

Table 5. Analysis of covariance (ANOCOVA) table of the yield in Egan, Tadian demo farm

S.V.	Y adjusted for X				F-tabular	
	d.f.	SS	MS	F-value	0.05	0.01
Error	11	-11526795.60	-1047890.50			
Treatment + error	17	3261428.57	191848.74	-0.183081		
Treatment adjusted	6	14788224.20	2464704.00	-2.352063 **ns**	3.09	5.07

ns not significant

Table 6. Analysis of variance (ANOVA) table of the yield in Ambasing, Sagada demo farm

S.V.	d.f.	SS	MS	F-value	F-tabular	
					0.05	0.01
Block	2	9902.38	4951.19			
Treatment	6	182445.24	30407.54	0.25 **ns**	3	4.82
Error	12	1437747.62	119812.3			
Total	20	1630095.24				

ns not significant

c.v. = 52.540

Table 7. Analysis of variance (ANOVA) table of the yield in Amgayang, Tadian demo farm

S.V.	d.f.	SS	MS	F-value	F-tabular	
					0.05	0.01
Block	1	11428.57	11428.57			
Treatment	6	221171.43	36861.91	2.23 **ns**	4.38	8.47
Error	6	99171.43	16528.57			
Total	13	331771.43				

ns not significant

c.v. = 24.656

The result, thus, imply that the standard strain of rhizobium as well as the local population of rhizobium in the three field were efficient in affecting the yield of beans. Using the standard strain alone revealed that a comparable yield with the application of inorganic fertilizer could be obtained (Table 8). Yield, though, is not a good measure of the symbiotic effectiveness and dinitrogen-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 8. Means of the yield (g) of beans in the three de mo farm

Treatments	Egan	Amgayang	Ambasing	
			1st harvest	2nd harvest
T0 – farmer's practice, FP (unlimed), control	600.000	545.000	733.333	583.333
T1 – farmers'practice, FP (limed)	966.666	775.000	516.666	600.000
T2 – BNF	433.333	350.000	733.333	416.666
T3 – BNF + FP	1016.666	525.000	678.333	533.333
T4 – BNF + bean fertilizer requirement	700.000	400.000	616.666	450.000
T5 – soil test kit analysis (STK)	1400.000	500.000	783.333	466.666
T6 – bean fertilizer requirement	400.000	555.000	550.000	400.000

<u>BNF technology and other agricultural practices</u>. Results revealed that the BNF technology is comparable with other agricultural practices including the farmer's practice (Table 5, Table 6, and Table 7). However, in economic terms, the use of inoculant for bean inoculation is cheaper than the use of inorganic fertilizers. Moreover, it is environmentally friendly and can be utilized for the succeeding cropping of the same legume crop because of their ability to survive in the soil and compete with an already existing population of rhizobium, which are either less infective or effective, provided the time interval is not a year or more.

Although, the data on yield is quite erratic simply due to no defined trend observed (Table 8), statistical analysis showed that there was no significant difference on the treatment means of the agricultural practices tested (Table 5, Table 6, and Table 7). 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.

The BNF technology as treatment 2 generally has lower yield than the farmer's practice or treatment 0 (Table 8), 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 (Table 8). Nodulation, however, in the farmer's practice either limed or unlimed is generally lower than that of the other practices (Table 4).

CONCLUSION

1.) In Egan, Tadian, there is a need for inoculation; whereas, 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 standard strain *Rhizobium leguminosarum* bv. *phaseoli* (CIAT) obtained from BIOTECH, UPLB, may be used as source of inoculant in the places selected as demo farms—Tadian and Sagada, because of its ability to adapt

with the existing climatic condition and survive and compete with the local population of rhizobium.

3.) BNF technology is still of advantage compared to other agricultural practices in the cultivation of beans primarily due to cheaper cost of the inoculant compared to inorganic fertilizers, and its being environmentally friendly.

RECOMMENDATIONS

1.) More demo farms or trials should be made to assess the need for inoculation and extend to farmers the new 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.) In the absence of other standard strains like CIAT, TAL may be used as long as the rhizobium contained in the package match the legume crop, because of specificity in host-rhizobium relationship.
3.) BNF technology should be widely disseminated to farmers as a way to encourage organic farming and decrease the cost of farm inputs because of comparable yield between the technology and farmer's practice.

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