Plant and Soil **204:** 107–115, 1998.

Analysis of *Phaseolus–Rhizobium* interactions in a subsistence farming system

W.S. de Oliveira[1], L.W. Meinhardt[1], A. Sessitsch[2] and S.M. Tsai[1,*]
[1]*Centro de Energia Nuclear na Agricultura, University of São Paulo, Caixa Postal 96, Piracicaba CEP 13400-970, São Paulo, Brazil and* [2]*FAO/IAEA Agriculture and Biotechnology Laboratory, Soils Science Unit, A-2444 Seibersdorf, Austria*

Key words: celB, *gusA*, *lac*Z, landrace, lime, molybdenum, nitrogen fixation, *Phaseolus vulgaris*, *Rhizobium tropici*, X-gal, X-gluc

Abstract

Poor bean yields in the Cunha region of the Mata Atlântica ecosystem in the state of São Paulo, Brazil, are associated with low agronomic inputs, plant disease, and soil erosion. To identify sustainable farming practices that increase production and maximize biological N_2 fixation (BNF), the effects of soil fertility and plant cultivar on seed yield and root nodule formation were measured under standard agronomic practices. Results from 16 sites showed that fertilizing with lime and molybdenum increased seed yields to 370% for the landrace Serro Azul. In addition to increased yields, plants grown with fertilizer had more nodules. Marked strains of *Rhizobium tropici* were tested under controlled environments. An indicator strain of *Rhizobium* containing the *gusA* marker gene was used. Our results verify that the indicator strain CM-255 GusA^{+}Hup^{+} had a high capacity to associate with the five bean varieties tested. Fertilization with P, K, S + micronutrients and liming were essential for better nodulation by the indicator strain. Under low fertility conditions, the landrace variety Serro Azul was poorly nodulated, when associated with native strains or with the indicator strain. However, under better soil fertility conditions, nodulation of Serro Azul by the marked *Rhizobium* strain was increased. The commercial variety Carioca 80SH showed no increase in nodulation (nodule number).

Introduction

It is common in tropical forest ecosystems to apply the slash-and-burn farming technique. Although this farming system is not sustainable, it is widely employed by small landholders. In Northeastern São Paulo, Brazil, there is an area of the Mata Atlântica ecosystem, the Cunha region, which takes its name from the small city near its center. Many of the farmers in the region have a subsistence lifestyle because of the poor soils, low yields and plant diseases that are endemic to the area. Traditionally, slash-and-burn techniques are used to farm this mountainous region. Farmers normally use a corn/bean intercropping scheme and the typical size of each farm is approximately ten hectares, with two hectares under production. The small lowlands in the region are farmed continuously, while the mountain slopes under production are rotated to another section every three to four years by cutting and burning the forest. The mountains normally have a slope of 45° and more, resulting in an additional problem, erosion. Commonly, two crops are planted each year. Especially because of the need to cut and burn new forest every few years, the Mata Atlântica ecosystem is subject to increased deforestation, a subsequent loss of soil fertility due to an increased rate of soil erosion and the loss of genetic resources.

* FAX No.: (055) 19-429-4610. E-mail: tsai@cena.usp.br
Plant and Soil is the original source of publication of this article. It is recommended that this article is cited as: *Plant and Soil* **204**: 107–115, 1998.

A considerable amount of information has been published regarding the influences and interactions of soil fertility and plant nutrition upon nodulation and nitrogen fixation (Cassman et al., 1981; Hernandez and Focht, 1985; Lynd and Ansman, 1989, 1990). Gates and Wilson (1974) were the first to find the stimulatory effect of mineral N (NH_4NO_3) on soybean, at rates as high as 60 mg kg^{-1} of substrate, in the presence of high levels of P. Gates and Müller (1979) also showed that mineral N can stimulate soybean nodulation in the presence of high levels of P and S, however, imbalanced nutritional conditions of N, P and S affect this trait dramatically. In addition, Lynd and Ansman (1989) found decreases in nodule numbers of peanuts when K was applied alone, but not when K was associated with the addition of P and Ca. Potassium was required in greater quantities than any other mineral in the soil. Dry matter and N accumulation in the legumes are extraordinarily responsive to K fertilization (Duke and Collins, 1985), accompanied by concomitant increases in nodule mass and number (Barta, 1982; Collins and Duke, 1981). Our previous work on interactions of N fertilizer and soil fertility also confirmed similar findings for common bean, and showed a synergistic effect on nodulation and nitrogen fixation, under high soil fertility conditions with amendments of P, K, and S plus micronutrients (Tsai et al., 1993). Molybdenum is essential for plant growth, and is required in two key enzymes associated with N assimilation: nitrate reductase and nitrogenase. Mo requirements of legumes relying on N_2 fixation are high and Brodrick et al. (1992) determined that Mo levels effected changes in bean seed size, and increases in shoot, root and nodule dry weights. Variations in the response to Mo were due to genotypes and growth conditions. Plants grown under low Mo showed a reduction in the seed Mo content by 83–85% and seed production by up to 38%.

Sustainable farming systems rely upon and promote conditions that minimize plant diseases, maximize nitrogen fixation by leguminous plants, maintain optimal soil pH and increase soil fertility. The long-term goals of this study were to understand the *Rhizobium*-fertility complexes in the region, and to identify traits of the plant–microbial interactions that affect biological N_2 fixation in the common bean varieties most commonly used by the farmers. Ultimately, the knowledge acquired in this study will be used to promote sustainable farming in the region.

Material and Methods

Soil fertility status in the area. Soil pHs in the region are acidic and range from 5.35 to 4.15, with the average being 4.59 (Table 1). Twelve of the sites have soils that are rated high in Al, while 9 are low in Ca (Table 1). The two sites rated as average for Al were also average for Ca. Most of the sites were rated low to very low in phosphorus. It was noted that, sites with low Ca had low S and those with high Ca had high S. Most of the sites were high in organic matter. Soil organic matter can function as a buffer to aluminum toxicity (Thung, 1991). Soils in the region are classified into three main types: gravelly podzolic, ortho red-yellow podzolic, or red-yellow latosol terrace phase. These soil types are primarily forest soils with low base saturation and are generally associated with low soil fertility (Brady, 1984).

Experimental design

Greenhouse experiments

Strains of R. tropici. The *Rhizobium tropici* strain CM-255 was selected by Narravo et al. (1993) for its favorable attributes. This strain is Hup$^+$, acid-tolerant, capable of nodulating at high soil temperatures and is an efficient nitrogen-fixing strain. The other *R. tropici* strain UM1899 (CM-01) (Graham, 1983; Martinez-Romero et al., 1991), is Hup$^-$, acid and high temperature-tolerant.

Marking Rhizobium *with* gusA *and* celB *genes.* We followed procedures for marking the *R. tropici* strain CM-255 with the *gusA* gene from a GUS Gene Marking Kit (Sessitsch, 1995). For studies with two markers – *gusA* and *celB* genes, *R. tropici* UM1899 (CM-01) was labeled with *celB*. Rhizobia expressing the *celB* gene can utilize X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranos ide) and those expressing *gusA* can utilize X-gluc (5-bromo-4-chloro-3-indolyl-β-D-glucuronide). Figure 2 shows a photograph of the colored Gus-marked and Cel-marked nodules recovered from beans grown in Leonard jars. Roots were treated with X-gluc and X-gal as described by Sessitsch (1995).

Field experiments at the Cunha region

Experimental plot design. Plots were established on 16 farming sites in the Cunha region. Each plot measured 14 × 14 m, with four subplots of 7 × 7 m each,

Table 1. Results from the chemical analyses from soil samples collected at 16 sites in the Cunha region

Sites	pH	P	K	Ca^{2+}	S	H^{+}, Al^{3+}	% Organic matter
1	4.65	7.7	0.08	1.26	1.8	4.01	2.17
2	5.15	13.1	0.29	2.37	3.2	3.08	3.03
3	4.65	20.7	0.33	1.39	2.0	5.22	2.54
4	5.35	13.7	0.53	3.24	4.6	3.25	3.16
5	4.45	5.0	0.13	1.36	1.9	6.79	3.40
6	4.70	102.3	0.13	1.19	1.8	7.95	4.09
7	4.20	5.6	0.41	4.07	5.2	6.79	3.34
8	4.70	29.0	0.19	1.00	1.4	10.91	3.71
9	4.20	20.5	0.15	1.88	2.7	12.78	3.16
10	4.70	10.6	0.19	0.70	1.2	22.80	5.57
11	4.55	5.2	0.12	0.40	0.7	18.47	2.91
12	4.30	4.7	0.40	1.15	1.9	8.84	4.45
13	4.40	4.1	0.14	0.28	0.5	20.52	2.42
14	4.60	2.9	0.13	0.88	1.3	13.47	1.79
15	4.75	7.4	0.09	0.61	1.0	7.16	3.34
16	4.15	4.3	0.15	1.28	1.8	4.01	2.66

Measurements in $cmol/dm^3$ of soil. H^{+} + Al levels below 3.50 are considered low, and above 6.00 are high.
% Organic matter above 2.50 is considered high, below 2.00 is low.

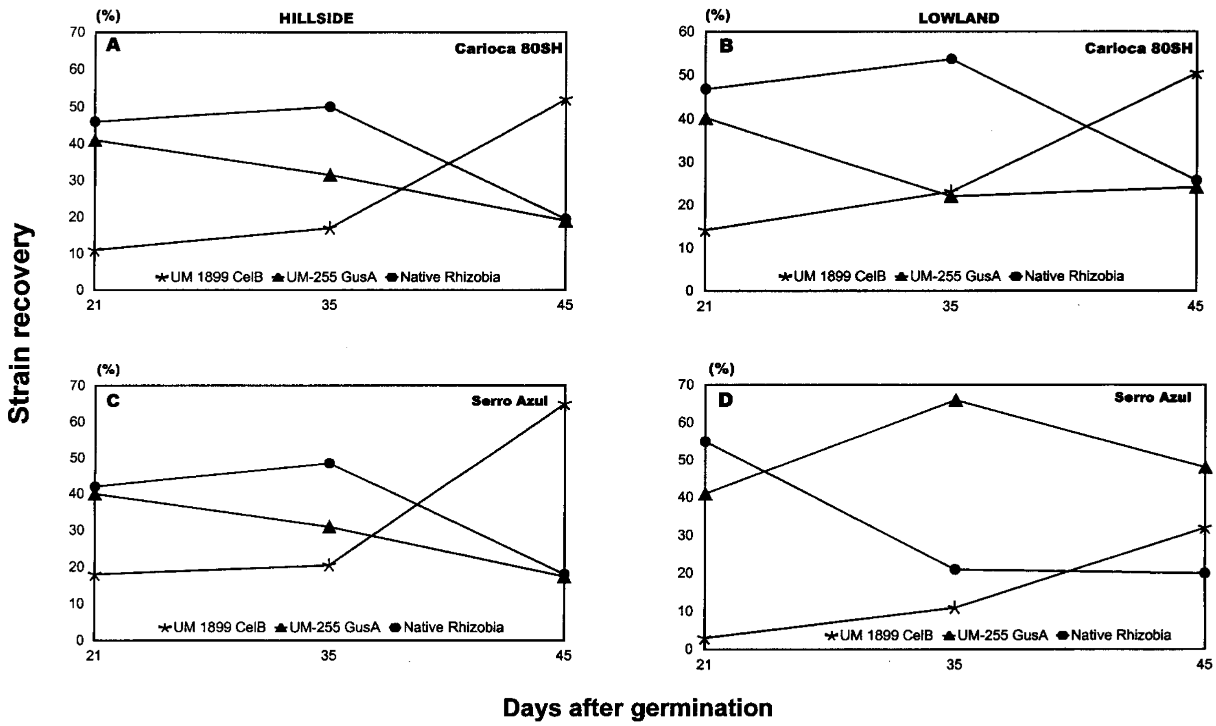

Figure 1. The identification of nodule-forming *Rhizobium* strains in two cultivars, from hillside and lowland soils of Cunha. Carioca 80SH is a commercial variety while Serro Azul is the most cultivated landrace in Cunha. Visualization of the dual inoculations was possible by using two substrates X-gluc and X-gal.

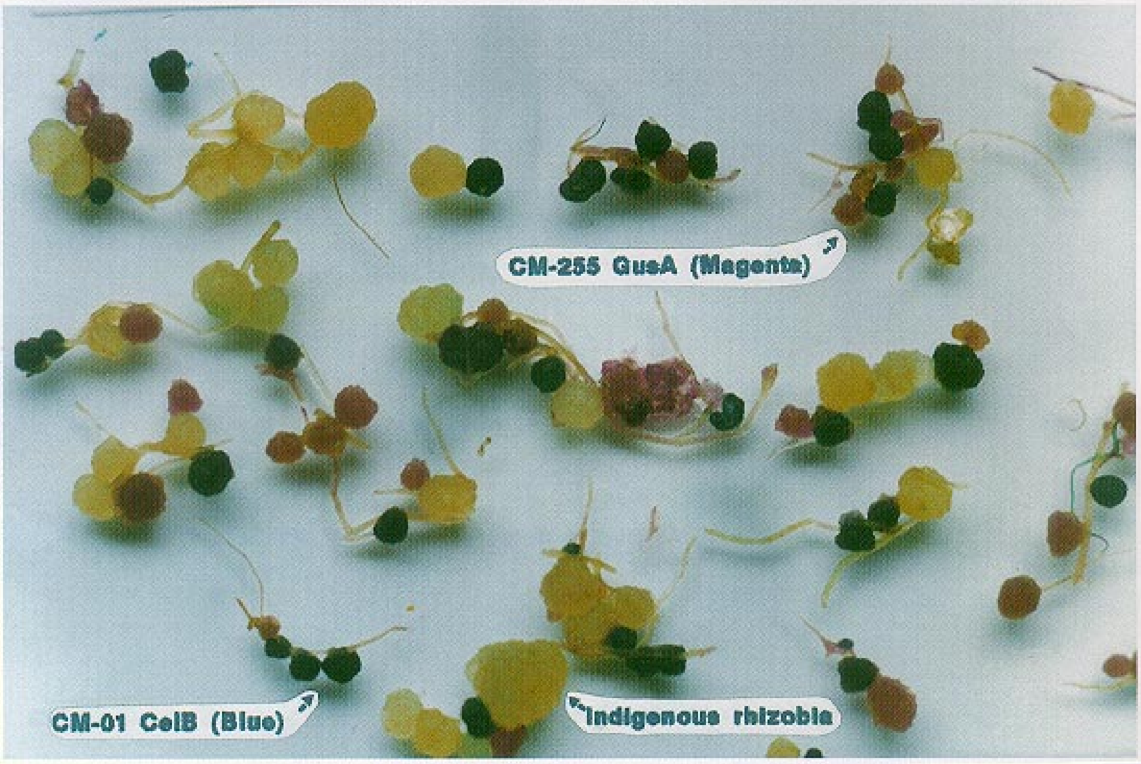

Figure 2. Photograph of the colored nodules, showing recovery of the introduced strains. X-gluc = cm-255 (*gusA*, X-gal = UM 1899 (*celB*, blue).

with four treatments randomly distributed within the subplots. Seeds were planted in rows spaced approximately 40 cm apart and the plants spaced 5 cm apart. Planting rate was 400 000 plants per ha.

Soil samples were taken and several soil amendments were applied to the experimental sites. Fertilizers were limited to nitrogen (N), (phosphorus) P, (potassium) K and lime providing (calcium) Ca and (magnesium) Mg. Dolomite PRNT 90.1 (lime) was used to buffer the soil pH, to increase soil Ca and Mg content and soil pH, which in turn can reduce the availability of Al and hence its toxic effects on the plants (Thung, 1991). Ammonium molybdate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, at 40 g/ha was applied twice (25 and 45 days after planting) as foliar sprays (Vieira, 1994). Fertilizer used in the experiments was at a rate of 200 kg/ha. Based on data from soil samples, the sites that were especially low in P and K were amended with additional amounts of P_2O_5 and KCl_2.

Nodulation evaluation – field trials. Nodule numbers were evaluated at 45 days after sowing. Soils were collected from sites 14 (from the hillside) and 15 (from the lowland) and were taken for greenhouse and lysimeter experiments.

Statistical analysis. The experimental design used a random distributed block design with 16 sites and 4 treatments. The Test for Multiple Comparisons (Tukey) was used for all the data obtained.

Results and discussion

By improving the fertility of the soil and correcting pH imbalances in the rhizosphere, we hoped that the efficiency of nodulation could be increased, thereby improving nitrogen fixation. Data collected in the region suggests that nodule number is affected by fertilization (Table 2). Also, adequate calcium levels and soil pHs are extremely important for the nodulation process, since low calcium and acid conditions inhibit nodule formation (Thung, 1991).

Table 2. Evaluation of rhizobia under controlled conditions – Leonard jars. Nodulation, plant biomass and total N accumulation in response to the inoculation of three strains of *Rhizobium tropici* (two commercial strains CM-255 and UM1899 and the native isolate Cunha-1) and the N control treatment. Plants were grown in vermiculite for 45 days

Treatment	Nodules (per plant)	Biomass (g/plant)	Total N (mg N/plant)
N – 10 mM	–	4.14A	12.4A
Cunha-1	234.97a	2.74B	9.01AB
CM-255	125.13b	2.91B	9.77AB
UM1899	227.59ab	2.32B	6.55B
C.V. (%)	28.80	30.40	32.90

Values are an average from four bean varieties – Carioca 80SH, Serro Azul, Serro Azul B, Serro Azul M.
Mean values followed by the same capital letters do not differ significantly at $P < 0.01$ by the Tukey test.
Mean values followed by the same lower case letters do not differ significantly at $P < 0.05$ by the Tukey test.

Nitrogen is a key component of soil fertility and may be introduced into an environment from nitrogen fixation or from nitrogen fertilizers. In this particular ecosystem, nitrogen comes from either the native leguminous species affiliated with the forest or from the production of beans. Environmental conditions such as soil types, fertility, pH, and bacterial efficiency all play an important role in determining the total fixed nitrogen in a system.

N_2 fixation and efficiency (Leonard jars). Under controlled conditions, a strain isolated from a Cunha soil (Cunha-1) was able to form a high number of nodules per plant. This would appear to be a highly favorable trait. However, total nitrogen in the shoot biomass was not affected by the increase in nodule number. The strain Cunha-1 appears to be slightly delayed in its ability to nodulate. At 21 days after germination the number of nodules derived from Cunha-1 was only one third the number of UM1899 or CM-255 (data not shown). At 45 days after germination, the nodule number was equal to or greater than UM1899 or CM-255. Wolyn et al. (1989) observed that the ability of rhizobia to nodulate in later developmental stages may lead to increased amounts of fixed nitrogen translocated to the pods.

Strain competition studies and use of marked strains with gusA *and* celB *(Leonard jars).* The concentration of *Rhizobium* cells in the soils of Cunha were 10^3 to 10^4 CFU/g soil, which is not sufficient to compete with inoculated strains (first sampling period 21 days, see Figure 1). However, at the second sampling period (35 days), native *Rhizobium* began to occupy a higher percentage of the nodules. At the third sampling (45 days), the percentage of nodules occupied by native *Rhizobium* had drastically changed and in most cases these organisms dominated nodule occupancy. Also, at the third sampling the color intensity of the various marked strains was lower than at previous times. Streit et al. (1995) observed similar differences in color intensity and found this to be associated with bacterial senescense.

The trial with cultivars in Leonard jars showed a significantly higher number of nodules and a higher level of nitrogenase activity for the Serro Azul M (Table 3). However, these increases were not reflected in the total nitrogen content. In a study by Pereira *et al.* (1984), 339 genotypes of bean were evaluated, and Carioca 80SH was characterized as having a high capacity to produce biomass. Therefore, the landrace varieties appear to be similar to Carioca 80SH for biomass accumulation.

Semi-controlled conditions (hillside × lowland soils from Cunha, in lysimeters). The introduction of different strains of *Rhizobium* into the soil under semi-controlled conditions, via seed inoculation, was not

Table 3. Evaluation of bean varieties under controlled conditions – Leonard jars. The landrace variety Serro Azul and its two variants Serro Azul B (Brilliant) and Serro Azul M (mottle), were compared to the commercial cultivar Carioca 80SH and to a N control treatment of 10 mm NH_4NO_3. Plants were grown in vermiculite for 45 days

Treatment	Nodules (plant)	N-ase* (μmol C_2 H_2/ plant/h)	Biomass (g/plant)	Nitrogen in dry matter (%)	Total N. (mg N/ plant)
Serro Azul B	181.21 B	11.65 AB	3.18	3.01	10.37
Serro Azul M	300.91 A	19.55 A	3.49	3.25	10.48
Serro Azul	187.08 B	10.95 AB	3.69	3.11	8.40
Carioca 80SH	180.54 B	6.26 B	2.74	3.30	8.47
N – 10mM	–	–	4.14	2.99	12.41
C.V. (%)	28.80	69.60	30.40	9.70	32.90

Data are the average of each variety in association with three different rhizobia strains.
Mean values followed by the same capital letters do not differ significantly at $P < 0.01$ by the Tukey test.
* Nitrogenase activity.

Table 4. Evaluation of rhizobia under semi-controlled conditions – 40 kg-soil containers. Nodulation, plant biomass and nitrogen accumulation were analyzed for three *Rhizobium tropici* strains, UM1899, CM-255 and Cunha-1. Native rhizobia present in the soil were also analyzed. A N control treatment (60 kg N/ha, supplied as NH_4NO_3) was also included. Data were collected 45 days after germination

Treatment	Nodules (per plant)	Plant: Biomass (g/plant)	Plant: Total N (mg N/plant)
N Control (60kg/ha)	–	8.11 A	184.65 A
Control	129.66 B	6.29 B	162.31 AB
CM-255	157.47 A	6.13 B	145.29 B
UM1899	140.19 AB	5.75 B	151.21 AB
Cunha-1	129.06 B	6.03 B	156.01 AB
C.V. (%)	22.8	23.4	25.6

Mean data of four replicates.
Values are an average from 4 varieties of beans, Carioca 80SH, Serro Azul, Serro Azul B, Serro Azul M.
Mean values followed by the same capital letters do not differ significantly at $P < 0.01$ by the Tukey test.

significantly different from the controls (Table 4). This could be due to the presence of highly competitive native strains of rhizobia. Vieira (1994) found that under improved soil conditions, native rhizobia may be more competitive than the introduced strains.

Under semi-controlled soil conditions, the landrace and commercial cultivars were compared for plant biomass, total nitrogen content, nitrogenase activity and nodule number (Table 5). While nodule numbers and nitrogenase activities were not significantly different, variation was found in biomass ac-

Table 5. Evaluation of bean varieties under semi-controlled conditions – 40 kg-soil containers. Landrace variety Serro Azul, its two variants Serro Azul B (brilliant) and Serro Azul M (mottle), were compared with the commercial cultivar Carioca 80SH. A 60 kg N/ha (supplied as NH_4NO_3), control was also included. Analyses were performed 45 days after germination.

Treatment	Nodules (per plant)	Nase* (μmol C_2H_2/ plant/hr)	Biomass (g/plant)	Nitrogen in dry matter (%)	Total N (mg N/ plant)
Serro Azul B	150.20 ns	9.28 ns	7.90 A	2.53 ns	198.81 A
Serro Azul M	137.81	9.91	6.03 B	2.46	147.36 B
Serro Azul	132.94	13.24	7.07 AB	2.48	175.82 AB
Carioca 80SH	135.00	8.26	4.85 C	2.46	117.58 C
60 kg N/ha	–	–	8.10	2.23	184.65
C.V. (%)	22.80	23.40	8.90	25.60	25.90

ns = not significant.
Means data of four replications. Values represent the average results of the variety in association with 3 different rhizobia strains Cunha-1, CM-255 and UM1899.
Mean values followed by the same capital letters do not differ significantly at $P < 0.01$ by the Tukey test.
* Nitrogenase activity.

Table 6. Field evaluation of bean varieties. Nodulation and plant biomass comparisons at 45 days and yield responses of the landrace Serro Azul and Carioca 80SH (commercial) to Mo and liming. Mean data are averages for the 16 sites

	Plant biomass (g m $^{-1}$)	Nodule number (per plant)	Yield (kg ha^{-1})
Cultivar			
Carioca 80SH	57.16*	41.1 ns	1375 ns
Serro Azul	50.26	39.7	1242
Liming			
4 ton ha^{-1}	61.59*	52.9**	1415 ns
0	50.26	31.9	1201
Molybdenum			
40 g ha^{-1}	53.73 ns	47.1 ns	1451**
0	50.07	42.9	1165
C.V. (%)	30.20	46.00	31.10

ns = not significant.
* Mean values different among themselves at a $P < 0.05$ level by F-test.
** Mean values different among themselves at a $P < 0.01$ level by F-test.

cumulation and in total nitrogen content. These observations suggest that the landrace varieties are better adapted to the Cunha region.

Field evaluation (16 sites at Cunha). Major factors limiting bean production in the Cunha area are low soil pH and low nutrient levels. Studies under field conditions showed (Table 6) that when nutrient deficiencies, such as that of P, K and S, are corrected, along with soil pH improvements through liming, yield can be increased significantly from 400 kg/ha to 1300 kg/ha. According to Graham et al. (1982) and Thung (1991), improvements of tropical soil pHs through liming may lead to better nodule formation as well as an increase in nodule number. In addition, under balanced soil nutrient conditions, Tsai et al. (1993) found positive effects on BNF traits by adding small amounts of nitrogen at early stages of the bean nodulation process.

The availability of Mo, an essential micronutrient for the symbiosis, is extremely limited in acid soils (Franco and Day, 1980). Addition of Mo, via foliar application, may correct the deficiency in the plant and enhance grain yield (Table 6). Vieira (1994) found that molybdenum when applied via foliar application, did not significantly increase the costs of bean production, since only small amounts of ammonium molybdate are necessary, and this may be applied in combination with foliar sprays for disease control.

Our data suggest that bean yield on the 16 sites from Cunha can be enhanced substantially by increasing soil fertility. Increased yields are extremely beneficial to the farmers in the region, and with proper management these yields could be sustainable. Sustainable crop production would reduce the amount of land used and decrease the extent of slash-and-burn farming, thereby allowing the possibility of returning much of the land to a natural state.

Acknowledgements

This contribution was supported by PADCT/CNPq, FAPESP and IAEA research grants. The authors would like to thank CNPq/RHAE for funding L W Meinhardt, CNPq for S M Tsai and CAPES for W S de Oliveira.

References

Barta A L 1982 Response of symbiotic N_2 fixation and assimilate partitioning to K supply in alfalfa. Crop Sci. 22, 89–92.

Brady N C 1984 Soil formation, classification and survey. *In* The Nature and Properties of Soils. 9th ed. MacMillan Publishing Co, New York.

Brodrick S J, Sakala M K and Giller K E 1992 Molybdenum reserves of seed, and growth and N_2 fixation by *Phaseolus vulgaris* L. Biol Fertil Soils 13, 39–44.

Cassman K G, Whitney A S and Fox R L 1981 Phosphorus requirements of soybean and cowpea as affected by mode of N nutrition. Agron. J. 73, 17–22.

Collins M and Duke S H 1981 Influence of potassium-fertilization rate and form on photosynthesis and N_2 fixation of alfalfa. Crop Sci 21, 481–485.

Duke S H and Collins M 1985 Role of potassium in legume dinitrogen fixation. In: Potassium in Agriculture. Ed. Robert D, Munson. Am. Soc. Agron. pp 443–466.

Franco A A and Day J M 1980 Effects of lime and molybdenum on nodulation and nitrogen fixation of *Phaseolus vulgaris* L. in acid soils of Brazil. Turrialba 30, 990–1005.

Gates C T and Müller W J 1979 Nodule and plant development in the soybean, *Glycine max* (L) Merr.: Growth response to nitrogen, phosphorus and sulflur. Aust. J. Bot. 27, 203–215.

Gates C T and Wilson W J 1979 The interaction of nitrogen and phosphorus on the growth, nutrient status and nodulation of *Stylosanthes humilis* H.B.K. (Townsville Stylo). Plant Soil 41, 325–333.

Graham P H, Viteri S E, Mackie F, Vargas A A T, Palacios A 1982 Variation in acid soil tolerance among strain of *Rhizobium phaseoli*. Field Crops Res. 5, 121–128.

Graham R D 1983 Effects of nutrient stress on susceptibility of plants to disease with particular reference to the trace elements. Adv. Bot. Res. 10, 221–276.

Hernandez B S and Focht DD 1985 Effects of phosphorus, calcium, and Hup^- and Hup^+ rhizobia on pigeon pea yields in an infertile tropical soil. Agron. J. 77, 867–871.

Lynd J G and Ansman T R 1989 Effects of P, Ca with four K levels on nodule histology, nitrogenase activity and improved 'spanco' peanut yields. J. Plant Nutr. 12, 65–84.

Lynd J G and Ansman T R 1990 Soil conditions with distinctive coralloid nodulation and nitrogen fixation of 'Mecca' alfalfa. J. Plant Nutr. 13, 77–94.

Martínez-Romero E, Segovia L, Mercante F M, Franco A A, Graham P H and Pardo M A 1991 *Rhizobium tropici*, a novel species nodulating *Phaseolus vulgaris* and *Leucaena* sp. Trees. Int. J. Syst. Bacteriol. 41, 417–426.

Navarro R B, Vargas A A T, Schröder E C and van Berkum P 1993 Uptake hydrogenase (Hup) in common bean (*Phaseolus vulgaris*) symbioses. Appl. Environ. Microbiol. 59, 4161–4165.

Pereira P A A, Araujo R S, Moreira R G, Steinmets S 1984 Capacidade de genótipos de feijoeiro de fixar N_2 atmosférico. Pesq. Agropecu. Bras. 19, 811–815.

Sessitsch A 1995 Manual for the Gus Gene Marking Kit. Joint FAO/IAEA Division, IAEA- Vienna, Austria. 37 p.

Streit W, Botero L, Werner D and Beck D 1995 Competition for nodule occupancy on *Phaseolus vulgaris* by *Rhizobium etli* and *Rhizobium tropici* strains can be efficiently monitored in an ultisol during the early stages of growth using a constitutive *gus* gene fusion. Biol. Fertil. Soils 27, 1075–1081.

Thung M 1991 Bean Agronomy in Monoculture in Common Beans: Research for Crop Improvement. Eds. A van Schoonhoven and O Voyset. CAB International, Wallingford.

Tsai S M, Bonetti R, Agbala S M and Rossetto R 1993 Minimizing the effect of mineral nitrogen on biological nitrogen fixation in common bean by increasing nutrient levels. Plant Soil 152: 131–138.

Vieira R F 1994 Effects of rhizobial inoculation, N and Mo applications on the nitrogen nutrition of common bean (*Phaseolus vulgaris* L.). Dr. Dissertation, ESALQ/USP-Piracicaba – S.P., 189 p.

Wolyn D J, Attewell J, Ludden P W and Bliss F A 1989. Indirect measures of N_2 fixation in common bean (*Phaseolus vulgaris* L.) under field conditions: The role of lateral root nodules. Plant Soil 113, 181–187.