Response of common bean (*Phaseolus vulgaris* L.) to *Rhizobium* inoculation and nitrogen fertilization

Axel Omar Ramírez-Madera

A thesis submitted in fulfillment of the requirements for the degree of

MASTER OF SCIENCE
in
AGRONOMY

UNIVERSITY OF PUERTO RICO
MAYAGÜEZ CAMPUS
2009

Approved by:

James S. Beaver, Ph.D. President, Graduate Committee	Date
David Sotomayor-Ramírez, Ph.D. Member, Graduate Committee	Date
Consuelo Estévez de Jensen, Ph.D. Member, Graduate Committee	Date
Miguel A. Muñoz-Muñoz, Ph.D. Chairperson of the Department	Date
Sonia Borges, Ph.D. Representative of the Graduate Studies	Date

Abstract

Common bean (*Phaseolus vulgaris* L.) producers need to use bean lines with improved nitrogen (N) use efficiency to decrease production costs. The goal of this thesis was to identify bean lines adapted to soils with low N availability. Four field experiments were conducted at the Isabela Substation in northwestern Puerto Rico on an Oxisol of the Coto series (very-fine Kaolinitic Isohyperthermic Typic Eutrustox). In the first experiment ninety-six bean lines were evaluated for yield and nodulation score in February 2007. Randomized Complete Block (RCB) experimental designs with 6 replications were used. Fertilization was 50 kg ha^{-1} as N, P_2O_5, and K_2O. Inoculation of 2.5 kg ha^{-1} of *Rhizobium leguminosarum* biovar *phaseoli* inoculant was applied at planting at a rate of 3 g of inoculant per 3.8 L of water. Nodulation scores were determined using the CIAT 1-9 scale where 1 = > 80 nodules and 9 = < 10 nodules plant^{-1}. Small red and black bean lines had the best nodulation scores and seed yields. The second and third field experiments were planted in June and October 2007. A split plot arrangement in a RCB design with 5 replications was used for both experiments. Whole plots were two N levels (0 and 25 kg ha^{-1}) and sub-plots were the best 38 bean lines selected from previous experiments. Lines with the best nodulation scores in the June 2007 field experiment were: Salagnac 90A (2.7), Arroyo Loro Negro (2.8) and PR 0427-7 (3.5). In general, the best nodulation scores were observed in the low N plots (0 kg ha^{-1}). There was a significant ($p<0.05$) and positive correlation (r=0.38) between the June 2007 nodulation scores and plant vigor scores from the October 2007 field experiment, which had a severe amount of root rot. A fourth field experiment

was planted under low N conditions in January 2008 using a RCB design with 5 replications. The lines with the best nodulation scores at 67 days after planting were: OAC Rico and PR 0427-7 with scores of 1.7 and 4.7, respectively. Lines PR 0340-3-3-1, PR 0443-151, and VAX 3, produced the greatest seed yields in the second and fourth field experiments.

Resumen

Los productores de habichuela (*Phaseolus vulgaris* L.) necesitan de variedades que puedan utilizar el N del suelo con mayor eficiencia para reducir los costos de producción. El objetivo de esta tesis es identificar líneas de habichuelas que puedan adaptarse a condiciones de bajo N en el suelo. Cuatro experimentos fueron realizados en la Subestación Experimental de Isabela de la Universidad de Puerto Rico. El suelo era un Oxisol de la serie Coto (very-fine Kaolinitic Isohyperthermic Typic Eutrustox). En el primer experimento se evaluaron 96 líneas de habichuelas en febrero de 2007. El diseño fue en bloques completos aleatorizados (DBCA) con 6 repeticiones. Se fertilizó a razón de 50 kg ha^{-1} de N, P_2O_5 y K_2O. La inoculación de 2.5 kg ha^{-1} del inoculante *Rhizobium leguminosarum* biovar *phaseoli* fue al momento de la siembra a razón de 3 g de inoculante por 3.8 L de agua. Se midió la nodulación a través de una escala del 1-9 del CIAT donde 1 = > 80 nódulos planta^{-1} y 9 = < 10 nódulos planta^{-1}. Las líneas rojo pequeño y negro fueron relativamente mejores en nodulación y rendimiento. El segundo y tercer experimentos fueron sembrados en Junio y Octubre de 2007. Para ambos experimentos, el diseño fue un DBCA en parcelas divididas con 5 repeticiones. Las parcelas completas fueron dos niveles de N (0 y 25 kg ha^{-1}) y las sub-parcelas fueron 38 líneas de habichuela seleccionadas de experimentos anteriores. En el experimento de Junio 2007, Salagnac 90A (2.7), Arroyo Loro Negro (2.8) y PR0427-7 (3.5) fueron las mejores líneas con buen valor de nodulación. El mejor valor de nodulación fue en 0 kg N ha^{-1}. Hubo una correlación significativa (p<0.05) y positiva (r=0.38) entre el valor de nodulación en

junio de 2007 y el valor del vigor de planta en octubre de 2007 (experimento que fue severamente afectado por una pudrición de raíz). El cuarto experimento fue sembrado en enero de 2008. Las condiciones fueron a un nivel bajo de N en el suelo en un diseño DBCA con 5 repeticiones. Las líneas con el mejor valor de nodulación a los 67 días después de siembra fueron: OAC Rico y PR 0427-7 con 1.7 y 4.7, respectivamente. Las líneas PR 0340-3-3-1, PR 0443-151 y VAX 3 produjeron el mejor rendimiento en el segundo y cuarto experimento.

Dedication

This thesis is dedicated to my family… especially my parents Ana Madera Torres and George Ramírez Ramírez, my sisters Yesi and Lory, and my love Jessica.

Acknowledgments

To God, who gave me life and the health I needed to fulfill this research. To my family, who taught me how to work hard and showed me how to love agriculture and nature. To Jessica, who always supported me.

To my partners at work: Ronald, Sonia, Gasner, and Luis. To my classmates: Nancy, Francisco, Luis, Edrick, Jorge “Pinolillo”, Gerson, Alexander, Ixia, Feliciano, and Edward.

To Dr. James Beaver for being the president of my committee and giving me the opportunity to complete a master’s degree. To Dr. David Sotomayor-Ramírez and Dr. Consuelo Estévez de Jensen for serving as a member of my committee, making corrections of this work, and giving me the guidance during my M.Sc. degree studies.

To all my professors, especially Dr. Raúl Macchiavelli, who helped me in the statistics and always gave me encouraging words. Dr. Linda Beaver, who was a mentor, and Dr. Miguel Muñoz, for giving me advice and his friendship.

To the Department of Agronomy staff, especially Gloria, Evelyn and Floripe, who always helped me and made me laugh. To the Isabela Experimental Substation staff, especially Alfredo, Seguinot, Erick, Kemuel, Sammy, and all who helped me in the field.

To the BNF lab (Dr. Eduardo Schroder, Prof. Myrna Alameda, Feliciano, Ixia, and Melissa) who helped me in the MPN method and supplied the equipment.

To Dr. Abner Rodriguez, Héctor and Ariel who helped on the Micro-Kjeldahl method, equipment, and reagents.

To Dr. Lydia Rivera, Luz Miriam and Verónica who helped me on the identification of *Macrophomina* and *Fusarium*.

To the Office of Graduate Studies, especially Lucy Serrano for her advice.

To all who helped me in one way or another and gave me the motivation to keep on going. I will be eternally grateful to all of you. Thanks!

Table of Content

List of Tables

List of Figures

List of Appendixes

Introduction

Common bean (*Phaseolus vulgaris* L.) is the second most important grain legume in the world based on cultivated area (Unkovich and Pate, 2000). Globally, the production of common bean is 18 million tons per year (generating $11 billion dollars in income) (Pachico, 1999). Common bean is fourth in importance as a source of protein in the tropical zone of America (Pachico, 1999). The common bean has three centers of origin: Mexico, Ecuador-Peru, and Argentina (Martínez-Romero, 2003) and was introduced to the Caribbean by Native Americans (Gepts and Debouck, 1991). The common bean is an important source of protein and energy in the traditional Puerto Rican diet. Green-shelled beans are produced locally by small-scale farmers, often in association with other crops such as plantains (*Musa* x *paradisiaca* L.) (Liu et al., 1998; Liu et al., 1999). During 2004-05, more than 500,000 kg of green-shelled beans were produced in Puerto Rico generating on income more than $1,000,000 at the farm level (Departamento de Agricultura de Puerto Rico, 2007). Snap beans are also produced on a limited scale to satisfy a niche market. Almost all of the dry beans (± 98%) consumed in Puerto Rico are imported. Therefore, there is a large potential market for locally produced beans.

The climate in Puerto Rico permits agricultural production throughout the year although the humid tropical climate requires the development of bean cultivars with resistance to disease and insect pressure. In addition, soils in the common bean production areas in Puerto Rico have low nitrogen availability. Soils range from basic to acidic and tend to have low organic matter content (Sotomayor-

Ramírez and Martínez, 2006), which is an important source of N in the soil. Consequently, increased biological nitrogen fixation (BNF) would be beneficial for common bean production in Puerto Rico.

Bean production in soils with low N availability benefits from the application of N fertilizer. The UPR Agricultural Experimental Station (AES) Technical Guide for Bean Production recommends an application of moderate amounts of N early in the growing season when bean plants have not nodulated (Beaver, 2006). The current recommendation of nitrogen application for common bean production in Puerto Rico ranges from 46 to 68 kg N ha^{-1} (Beaver, 2006). In heavy, humid, compacted or poorly aerated soils, a split application of nitrogen is recommended: the first before or during the planting and the second during flowering.

A symbiosis with the bacteria *Rhizobium* permits the common bean to fix N from the atmosphere and helps to replenish the soil N pool (Triplett et al., 2007; Zahran, 1999; Zurdo-Piñeiro et al., 2004). This mechanism increases seed yield and improves soil fertility over time. The presence of native strains of *Rhizobium* has been detected in soils of Puerto Rico (Velázquez-Báez, 1984). Native strains of *R. gallicum* and *R. tropici* in Puerto Rico are also capable of infecting common bean (Zurdo-Piñeiro et al., 2004). Fernández-Toledo (1996) isolated strains of *R. leguminosarum* bv. *phaseoli* and *R. tropici* from beans in Puerto Rico. Some authors reported that inoculation with *Rhizobium* strains improve nodulation and enhances BNF (Bliss, 1993b; Date, 2000; Giller and Cadisch, 1995; Moawad et al., 1998). Biological nitrogen fixation is enhanced with low soil N availability. Hardarson and coworkers (1993) made an extensive evaluation using a diversity of common bean genotypes in 7 different locations (Austria, Brazil, Chile, Colombia, Guatemala, Mexico, and Peru) under low N conditions (10 kg ha^{-1}). They reported a range of N derived from the atmosphere (%Ndfa) from 0 to 73% and a range of fixation from 0 to 165 kg ha^{-1}. It has been found that fertilization as little as 20 to 50 kg N ha^{-1} can suppress nodule formation and fixation activity in common bean (Da Silva et al., 1993; Saito et al., 1984; from Mangual-Crespo, 1987; Dean and Clark, 1980; Mangual-Crespo, 1987). Some researchers, however, reported that applications in the range of 22 to 33 kg N ha^{-1} can enhance both nodulation and seed yield (Mangual-Crespo et al., 1987; Müller et al., 1993; Tsai et al., 1993).

In Puerto Rico, limited research has been conducted concerning the response of common bean to *Rhizobium* inoculation and N fertilization. However, the use of N fertilization and inoculation and its effect on seed yield has been explored and results showed that inoculation with *Rhizobium* is an agricultural practice that favors nodulation and increased seed yield (Catano-Catano, 1990; Cordero-García, 1979; Fernández-Toledo, 1996; Mangual-Crespo et al., 1987; Velázquez-Báez, 1984; Velázquez et al., 1988). There is a need to give greater importance to the improvement of N fixation capacity, N acquisition and N use efficiency in common bean breeding programs (Santalla et al., 2001). Much of the research concerning BNF in other countries is orientated toward sustainable agriculture where farmers with limited resources have to produce common bean with minimum or no N fertilizer, while providing a high quality product to the consumer. "Biological N_2 fixation is an important aspect of sustainable and environmentally friendly food production and long-term crop productivity" (FAO, 2000). The results from this investigation are expected to benefit the bean research community due to the importance of the common bean as a crop in the

Americas (Central, South and Caribbean) and Africa (Hungria and Vargas, 2000; Moawad et al., 1998; Unkovich and Pate, 2000).

Objectives

1. Identify common bean lines that are better adapted to low N soils.
2. Estimate the BNF capacity of these breeding lines based on the nodulation score, dry matter production, N content, and seed yield.
3. Compare the BNF capacity of older and the more recently released bean cultivars.

Literature Review

Triplett et al. (2007) noted that “the largest single fossil fuel input into most crop production systems is nitrogen fertilizer”. Raun and Johnson (1999) reported that two thirds of the N applied to cereal production is lost and only one third is collected in the grain. Overuse of N fertilizers can cause environmental problems such as contamination of the ground and surface water. According to FAO (2000) and Jensen and Hauggaard-Nielsen (2003), excessive amounts of N fertilizer can contribute to the depletion of ozone by the effect of greenhouse gas CO_2. Moreover, N fertilizer is derived from ammonia which is produced from natural gas. Thirty-three million British thermal units (mm Btu) of natural gas is needed to produce 1 ton of ammonia. From 2000 to 2006, the price of ammonia rose from \$227 to \$521 ton^{-1} (Huang, 2007). The increased transportation cost of ammonia and other N fertilizers is also a problem. This situation has resulted in increased production costs for farmers. Increased BNF and N use efficiency can reduce the levels of fertilizer N needed and reduce the potential for contamination. Some researchers have reported bean seed yields greater than 1,500 kg ha^{-1} by creating conditions favorable for BNF and applying a limited amount of N fertilizer (25 kg N ha^{-1} or less) (Kipe-Nolt et al., 1993; Mangual-Crespo et al., 1987; Manrique et al., 1993). In Brazil, the N fertilizer recommendation for bean production is an application of 20 kg N ha^{-1} at planting (Müller et al., 1993). Biological N fixation can be affected by edaphic factors such as pH or availability of nutrients, photosynthesis, climatic factors, and management of the legumes. These factors can influence legume seed yield, N fixation (FAO, 2000) and N reservoirs in the soil (Havlin et al., 1999).

Annual losses on agricultural land of 20-70 kg N ha^{-1} can be controlled by additions of N fertilizer and/or BNF (Bøckman, 1997; Giller and Cadisch, 1995; Jensen and Hauggaard-Nielsen, 2003; Zahran, 1999). Biological N fixation is a critical process for providing inputs of N in natural and agricultural systems, balancing the N cycle and buffering the losses in agriculture (Bøckman, 1997; Brady and Weil, 2002; Ishizuka, 1992; Jensen and Hauggaard-Nielsen, 2003; Lynch, 2007; Wani et al., 1995; Zahran, 1999). Peoples et al. (1995) point out that inorganic N from fertilizer is more susceptible to losses than N coming from legumes. They concluded that managing N derived from the atmosphere can complement and in some cases substitute N fertilizer. Peoples et al. (1995) noted that BNF improves the N economy of soils. There is an urgent need to greater utilize this process in modern farming systems (Giller and Cadisch, 1995; Graham and Vance, 2000). Nitrogen fixed biologically supply the major input of N to soils on the worldwide level (Bøckman, 1997). According to Ishizuka (1992), 17.2 x 10^7 tons per year is the total BNF in the world. Through symbiosis legumes can fix a minimum of 70 million metric tons per year globally (Brockwell et al., 1995). Some legumes can fix as much as 140 kg of N ha^{-1} per year, equal to 35 x 10^6 Mg of total N fixed worldwide (Brady and Weil, 2002). Bacteria can fix 25-80% of the total N in legumes (Graham and Vance, 2000; Havlin et al., 1999). Common bean has been reported to fix between 30 to 50 kg N ha^{-1} per year (Brady and Weil, 2002). It has been reported that common bean can fix from 22 to 90 kg N ha^{-1} per year in temperate climates (Havlin et al., 1999). In crop rotations, common bean harvested for grain can accumulate 200 kg N ha^{-1} per year, but large proportion of that is removed in the grain yield (Dawson et al., 2008). Bliss (1993b) observed that common bean can fix a minimum of 50 kg ha^{-1} and 40-50% of the plant N from fixation in adapted cultivars. Peoples and Herridge (2000) reported that nodulated roots can fix between 30 to 50% of the total N. The N fixed by nodules can be used by the host plant, become accessible to non-fixing plants growing in combination with legumes, or immobilized and incorporated in the soil organic matter (Brady and Weil, 2002; Havlin et al., 1999). These sources contribute greatly to agricultural management practices such as cover crops, crop rotation, intercropping, and multiple cropping by improving BNF, yield and sustainability of agricultural systems (Hungria and Vargas, 2000; Wani et al., 1995).

Nitrogen from BNF is used in the growth and development of plants as a component of amino acids and amides that form proteins, enzymes, and nucleic acids (DNA, RNA), nucleotides, hexoamines, etc. (Taiz and Zeiger, 2002). Nitrogen is also a component of the chlorophyll molecule and plays a role in carbohydrate utilization. Acquisition of nitrogen stimulates root growth and development, and absorption of mineral nutrients (Brady and Weil, 2002). The biomass of plants in general contains from 1 to 5% of N (Brady and Weil, 2002; Havlin et al., 1999). Nitrogen is deficient in most soils of the tropics resulting in reduced plant growth, chlorosis (yellowing of the leaves) and a low shoot to root ratio (Brady and Weil, 2002; Havlin et al., 1999; Taiz and Zeiger, 2002). Nitrogen fertilizer application is usually required because common bean can only fix a part of what it needs (Bliss, 1993b). The application of N fertilizer will enhance seed and tissue protein, crop yield and production of biomass.

Nitrogen fixation can be made by an industrial process called Haber-Bosch, which requires a large amount of energy and high temperature (400-650℃) and pressure (20-40 MPa) (Epstein and Bloom, 2005) and “non-renewable fossil fuels” (FAO, 2000). Meanwhile symbiotic N fixation can be done at ambient temperature and pressure (Epstein and Bloom, 2005) requiring less energy input (renewable carbohydrates produced by the plants) (FAO, 2000). The fertilizer industry fixes 77-80 x 10^6 Mg of N per year (Brady and Weil, 2002; Epstein and Bloom, 2005). Nitrogen fertilizer prices have increased greatly in the recent years because of the continuing increase in the cost of natural gas used in the Haber-Bosch process to produce ammonia. Ammonia prices increased 130% from 2000 ($227 per ton) to 2006 ($521 per ton) (Huang, 2007).

In Puerto Rico, limited research has been conducted with BNF of common bean. In 1984, Velázquez-Báez (1984) studied the effect of inoculation of *Rhizobium* on the performance of the bean cultivar ‘Arroyo Loro’. The treatment with the greatest seed yield (1,863 kg ha^{-1}) received 100 kg ha^{-1} of N as ammonium sulfate. Inoculation was not effective in increasing the number or the dry weight of nodules because a high number of *R. phaseoli* was detected in the soil. Applying N (20 and 100 kg ha^{-1}) did not increase the N concentration (%) in the stover or the bean seed. No differences between seed or soil inoculation were observed (Velázquez-Báez, 1984).

Mangual-Crespo et al. (1987) evaluated the effect of different levels of N and *Rhizobium* inoculation on the performance of the white bean cultivar ‘Bonita’ grown in the semi-arid southern region of Puerto Rico. They used six N levels: 0, 22, 45, 90, 180, and 360 kg ha^{-1}, supplemented with P, K, and Mg. *R. phaseoli* was tested in inoculated and un-inoculated plots. An increase in nodulation was found in the 22 kg N ha^{-1} level of fertilization as well as in the inoculated plots. They also reported a reduced number and nodule weight at the higher nitrogen fertilizer application rates. They concluded that 22 kg N ha^{-1} and inoculation with *Rhizobium* improved nodulation. Seed yield did not increase with the addition of N fertilizer or inoculation.

Another study was conducted in Puerto Rico with 24 accessions from the Pompadour red mottled bean landrace collection from the Dominican Republic and a non-nodulating soybean (*Glycine max* L. Merr.) (Catano-Catano, 1990). The application of N fertilizer reduced the number and weight of nodules although fertilization did increase yield and total N in the seed. Pompadour B and Pompadour E lines had the best BNF characteristics based on nodule number, nodule dry weight, shoot dry weight, grain yield, N concentration (%), and protein concentration (%) of the seed and total N yield (Catano-Catano, 1990).

Fernández-Toledo (1996) evaluated the BNF capacity of 30 common bean breeding lines to determine their ability to fix N under high temperature conditions. No N fertilization and no inoculation were applied to measure the effectiveness of native strains of *Rhizobium*. Soil *Rhizobium* strains were isolated during the warm season. *R. leguminosarum* bv. *phaseoli* and *R. tropici* were identified. The most promising lines, including XAN 176, 92BG402, Talamanca, and PR9350-35, produced mean seed yields greater than 1,000 kg ha^{-1} depending exclusively on BNF and N available in the soil (Fernández-Toledo, 1996).

In Peru, Manrique et al. (1993) evaluated the yield and BNF of 22 bean lines after inoculating the soil with a liquid mixture of five local strains of *Rhizobium* *leguminosarum* biovar. *phaseoli* and applying 10 kg N ha^{-1} after planting. The best cultivar for N_2 fixation in both seasons was 'Caballero' with the greatest biomass yield, N yield, %Ndfa, and fixed N_2, during both winter and summer seasons. Seed yield was measured only during the summer growing season in which 'Caballero', Bayo G-7.5-2, and 'Tortola Diana' produced more than 1,500 kg ha^{-1}. Cultivars 'Caraota Brasilano' and 'Blanco' had the best performance in N yield, %Ndfa, and fixed N_2 during the winter season. Some of the mutant lines of 'Bayo' and 'Canario' were good fixers during the summer season. Because there were different growth habits of common bean and differences in adaptation, seed yield was not correlated with N_2 fixation parameters (Manrique et al., 1993).

Da Silva et al. (1993) observed that N fertilization at low rates (10 kg N ha^{-1} at planting) stimulated nodule weight and nodule number in trials conducted in Brazil. Moreover, nodule weight and nodule number decreased at higher rates of fertilization (40 kg N ha^{-1}). Using an Acetylene Reduction Assay (ARA), they found a reduction in nodule mass and nodule activity when N was directly applied to the soil and an even greater reduction when rates were greater than 30 kg N ha^{-1} at an early stage of plant growth. A 57% increase in shoot biomass was found with the *Rhizobium* inoculation. Seed inoculation increased 123% of the N and 89% of the P accumulated in the seed. There was no effect on seed production with increasing rates of N applied directly to soil. When 10 kg N ha^{-1} was applied, BNF was higher in one site than the other. Using ARA they found lower BNF values at high N application rates. They recommended inoculation of *Rhizobium* in places where native rhizobia are not abundant (Da Silva et al., 1993).

In Brazil, Müller et al. (1993) conducted a greenhouse evaluation of the bean cultivars ‘Puebla 152’ and ‘Negro Argel’ at four levels of N (0, 2.5, 12.5 and 25.0 mg N kg^{-1} of substratum). The pots were inoculated with 0.4 mL of 10^8 cell of the *Rhizobium leguminosarum* biovar *phaseoli* strain CIAT 899. No cultivar differences in N accumulation were found. There was an addition of N by BNF of the two bean cultivars at the two mineral nitrogen application rates. The results suggested that additional BNF occurs after flowering (Müller et al., 1993).

In Costa Rica, Ramírez (1984) evaluated the effect of five N application rates (0, 50, 100, 150, and 200 kg ha^{-1}) and four rates of P (0, 40, 80, and 120 kg ha^{-1}) on the performance of common bean. Phosphorus was applied at planting. Half of the N application was applied at planting and the second half was applied four weeks later. The fungal pathogen (*Sclerotium rolfsii*) affected production. The fertilization rates of 200 kg N ha^{-1} and 120 kg P_2O_5 ha^{-1} produced the greatest seed yield (1,363 kg ha^{-1}).

Materials and Methods

Most Probable Number

The Most Probable Number (MPN) technique was used to determine the population density of rhizobia in the inoculant and the soil. The MPN was estimated using the methodology described in the Handbook for Rhizobia (Somasegaran and Hoben, 1994). Siratro (*Macroptilium atropurpureum*) seeds were scarified, incubated and planted in tubes with agarose (Somasegaran and Hoben, 1994). One seed was planted in each tube. The inoculations of diluted inoculant were 0.3 mL of tenfold dilutions (10^{-1}-10^{-10}) consisting of two replications. Four to six weeks after inoculation, data was collected on the presence (+) or absence (-) of nodules. Soil samples were taken from the Isabela Experimental Substation from soil near the experimental site that had not been inoculated. The first dilution was prepared putting 10 g of soil in 90 mL of sterile water. Four repetitions were used to estimate the MPN of the soil.

Initial field screening for nodulation and yield capacity

Five field trials were planted at the University of Puerto Rico Substation near Isabela, Puerto Rico. The Substation is located in northwest of Puerto Rico at 67.3° longitude and 18.3° latitude. The elevation is 128 m above mean sea level. The mean annual temperature is approximately 29.3℃ with a mean annual precipitation of 1524 mm (Junta de Planificación, 2006). Table 1 presents the means of monthly temperatures, wind speed and precipitation at the Isabela Experimental Substation from February 2007 to April 2008. The soil is an Oxisol of the Coto series (very-fine Kaolinitic Isohyperthermic Typic Eutrustox) and is characterized as an acidic clay soil with intermediate fertility (Junta de Planificación, 2006).

Table 1. Means of monthly temperatures, wind speed and precipitation at the Isabela Experimental Substation, Puerto Rico.

Month	Maximum temperature (℃)	Minimum temperature (℃)	Mean temperature (℃)	Wind speed (kmph)	Precipitation (mm)
February 07	27.9	19.1	23.6	7.6	52.6
March 07	27.6	19.7	23.5	7.9	254.5
April 07	27.9	20.5	24.2	8.9	146.8
May 07	29.1	21.5	25.1	5.0	281.9
June 07	30.0	22.3	26.1	7.1	27.9
July 07	30.2	22.7	26.4	8.7	68.6
August 07	30.0	23.0	26.2	8.7	111.8
September 07	29.7	21.8	25.5	6.0	154.9
October 07	29.6	21.7	25.1	5.3	121.9
November 07	27.8	19.8	23.4	6.6	193.0
December 07	26.1	19.0	22.7	10.5	157.5
January 08	25.4	17.8	21.7	9.0	53.8
February 08	25.8	18.6	22.3	12.1	45.2
March 08	26.1	18.1	22.2	12.1	28.4
April 08	24.6	16.1	20.6	10.1	130.0

The initial screening was five field experiments (black, small red, white, pinto cream and Andean origin bean lines) planted on February 1, 2007 during the dry and cool winter season. The field experiments were arranged in Randomized Complete Blocks (RCB) with 6 replications. The experimental units were single rows, 1 m in length, 0.6 m between rows, 10 seeds per row, and 0.1 m between plants. This series of experiments included bean lines of diverse origin, seed type and growth habit (Tables 2-6). The first trial included 27 black-seeded lines (Table 2). The second trial included 27 small red bean lines (Table 3). The third trial included 12 white-seeded lines (Table 4). The non-nodulating white bean line, R-

99 OAC Rico (Park and Buttery, 2006) was included in the trial as a control. Ten pinto and cream lines were evaluated in the fourth trial (Table 5). Twenty bean lines of Andean origin were included in the fifth trial (Table 6). The non-nodulating red mottled line NOD-125 (Pedalino et al., 1992) was included in the trial as a control (Bliss, 1993a; Catano-Catano, 1990; Fernández-Toledo, 1996).

Table 2. Black-seeded bean lines evaluated for nodulation score and seed yield in a field trial planted at Isabela, Puerto Rico in February 2007.

Line	Origin[1]	Growth habit[2]	Unique trait(s)
Porrillo Sintético	Mexico	I	
Talamanca	CR	I	Web blight resistance
ICTA Ostúa	Guatemala	I	
Río Tibagi		I	
PR0443-151	PR	I	Root rot resistance
Arroyo Loro Negro	PR	I	Web blight and rust resistance and heat tolerance[3]
DOR 390 Negro Tacana	Mexico	I	BGYMV resistance
UCR 55	CR	I	Anthracnose resistance
Negro INIFAP	Mexico	I	
DOR 500 Negro Tropical	Mexico	I	BGYMV resistance
INTA Precoz	Guatemala	I	Earliness
Black Rhino	US	I	
Jaguar	US	I	
PR0427-49	PR	I	BGYMV resistance
Midnight	US	I	
SEN 3	CIAT	I	Drought
SEN 21	CIAT	I	Drought
T-39	US	I	
Condor	US	I	
B05040	US	I	
B04554	US	I	
B05055	US	I	
B04316	US	I	
Aifi Wuriti	EAP	I	
MH-43-2	PR	I	Web blight resistance
PRF-9924-50N	EAP	I	
BCN-0202-94	EAP	I	

[1] Origin: CR=Costa Rica, EAP=Escuela Agrícola Panamericana, CIAT=Centro Internacional de Agricultura Tropical, PR=Puerto Rico, US=United States. [2] Growth habit: D=determinate and I=indeterminate. [3] Beaver et al., 2003.

Table 3. Small red bean lines evaluated for nodulation score and seed yield in a field trial planted at Isabela, Puerto Rico in February 2007.

Line	Origin[1]	Growth habit[2]	Unique trait(s)
Rojo de Seda	El Salvador	I	Commercial seed type
Sedona	US	I	
SRS 6-6	EAP	I	
Cardenal	EAP	I	
DEHORO	Honduras	I	BGYMV resistance
Macuzalito	EAP	I	
Pamichial 1	EAP	I	
Nueva Esperanza	EAP	I	
Amadeus 77	EAP	I	BGYMV resistance, seed type
Carrizalito	EAP	I	BGYMV resistance, seed type
Milineo	EAP	I	
CENTA Pupil	EAP	I	
Tío Canela 75	EAP	I	
VAX 3	CIAT	I	Root rot resistance, common bacterial blight resistance
Bribri (MD-23-24)	EAP	I	BGYMV, web blight and heat tolerance
DICTA 113	Honduras	I	
DICTA 122	Honduras	I	
SER 16	CIAT	I	Drought tolerance
DOR 364	Honduras	I	BGYMV resistance and heat tolerance
DICTA 17	Honduras	I	
RAB 655	CIAT	I	Low fertility tolerance
SER 26	CIAT	I	
VAX 6	CIAT	I	Common bacterial blight resistance and heat tolerance
Merlot	US	I	BCNMV and BCMV resistance
PR9771-3-2	PR	I	
SRS 56-3	EAP	I	
SEDA	El Salvador	I	

[1] Origin: CIAT=Centro Internacional de Agricultura Tropical, EAP=Escuela Agrícola Panamericana, PR=Puerto Rico, US=United States. [2] Growth habit: D=determinate and I=indeterminate.

Table 4. White bean lines evaluated for nodulation score and seed yield in field trials planted at Isabela, Puerto Rico in February 2007.

Line	Origin[1]	Growth habit[2]	Unique trait(s)
Matterhorn	US	I	
Weihing	US	I	
Morales	PR	I	bgm-1 gene for BGYMV resistance, I gene BCMV resistance and rust resistance
Vista	US	I	
PR0443-48 (Verano)	PR	I	BGYMV resistance
PR0301-181 (Beníquez)	PR	I	BGYMV and BCMNV resistance
BelMiDak RMR 10	US	I	Rust and BCMNV resistance
BelMiDak RMR 12	US	I	Rust and BCMNV resistance
R-99 OAC Rico (No-nod)	Canada	I	No nodulation
OAC Rico	Canada	I	
Seahawk	US	I	
PR0427-7	PR	I	BGYMV and Igm

[1] Origin: PR=Puerto Rico, US=United States. [2] Growth habit: D=determinate and I=indeterminate.

Table 5. Pinto and cream bean lines evaluated for nodulation score and seed yield in trials planted at Isabela, Puerto Rico in February 2007.

Line	Origin[1]	Growth[2]	Unique trait(s)
BAT 477	CIAT	I	Water stress tolerant
A 774	CIAT	I	Low soil fertility tolerance
ABCP-8	US	I	
Maverick	US	I	
Montrose	US	I	
Othello	US	I	
USPT ANT-1	US	I	Anthracnose resistance
USPT CBB-1	US	I	Common blight resistance
P04205	US	I	
Carioca	BR	I	Low soil fertility tolerance, root-knot nematode resistance

[1] Origin: CIAT=Centro Internacional de Agricultura Tropical, US=United States, BR=Brazil. [2] Growth habit: D=determinate and I=indeterminate.

Table 6. Andean bean lines evaluated for nodulation score and seed yield in field trials planted at Isabela, Puerto Rico in February 2007.

Line	Origin[1]	Growth habit[2]	Unique trait(s)[4]
Cardinal	US	D	
USCR 7	US	D	
PR0422-4 (T-3)	PR	D	
PR0422-28 (T-19)	PR	D	
PR0422-29 (T-20)	PR	D	
PR0422-30 (T-21)	PR	D	Common blight resistance
PR0422-39 (T-27)	PR	D	
PR0422-41 (T-28)	PR	D	
PR0422-57 (T-41)	PR	D	
Rosado	Panama	D	
PR9920-171	PR	I	Heat tolerance
Indeterminate Jamaica Red (IJR)	Jamaica	I	Heat tolerance
Redhawk	US	I	
Montcalm	US	D	
BRB-198	CIAT	I	BCMNV resistance
Salagnac 90A	Haiti	I	Low soil fertility tolerance
NOD-125 (no-nod)	CIAT	D	No nodulation
JB-178	Dom. Rep.	D	
PC-50	Dom. Rep.	D	Rust resistance
Rojo	Tanzania	D	

[1] Origin: CIAT=Centro Internacional de Agricultura Tropical, PR=Puerto Rico, US=United States.
[2] Growth habit: D=determinate and I=indeterminate.

All plots were fertilized with 50 kg ha^{-1} of N, P_2O_5, and K_2O. All lines were inoculated (Bliss, 1993b; Date, 2000; Giller and Cadisch, 1995; Moawad et al., 1998) with a commercial inoculant containing strains of *Rhizobium leguminosarum* biovar *phaseoli* of N-DURE® (INTX Microbials, LLC at Kentland, Indiana). N-DURE is made out of humus for dry beans and provided 2.895 million viable cells per gram (2.9x10^{6}, determined by the MPN method) of *Rhizobium leguminosarum* biovar *phaseoli*. According to Velázquez-Báez (1984) 10^{6} cells per g of inoculant is a low quantity of rhizobia in an inoculant. Inoculation of 2.5 kg ha^{-1} of inoculant was applied in a liquid suspension using a backpack sprayer at a rate of 3 g of inoculant per 3.8 L of water. It was applied to the open seed furrow after planting but before the seed was covered with soil. The field was maintained clean of weeds and sprinkler irrigation was applied when necessary.

The parameters nodulation score and seed yield were used to estimate the BNF capacity (Bliss, 1993a; Bliss and Miller, 1988; Fernández-Toledo, 1996; Mangual-Crespo et al., 1987; Müller et al., 1993; Unkovich and Pate, 2000). Two roots per experimental unit were extracted carefully using a shovel on March 19, 2007 (45 days after planting, DAP) to count nodules and estimate the nodulation scores (Catano-Catano, 1990; Da Silva et al., 1993). The nodulation scores were based on the CIAT 1-9 scale where: 1 (excellent) = more than 80 nodules plant^{-1}, 3 (good) = 41 – 80 nodules plant^{-1}, 5 (intermediate) = 21 – 40 nodules plant^{-1}, 7 (poor) = 10 – 20 nodules plant^{-1}, 9 (very poor) = less than 10 nodules plant^{-1} (Van-Schoonhoven and Pastor-Corrales, 1987). The field trials were harvested on April 23-25, 2007. Seed yield per experimental unit was converted to kg ha^{-1} (Da Silva et al., 1993). Results were analyzed using an Analysis of variance. Means of bean lines were compared using Fisher's LSD at 0.05 level of confidence.

Evaluation of promising bean lines for nodulation and yield capacity at low levels (0 and 25 kg ha^{-1}) of N fertilization

The second field evaluation for nodulation and yield was planted in the same field on June 6, 2007. Twenty four of the most promising lines from the previous trial were selected for further evaluation based on nodule scores and seed yield potential (Table 7). The new evaluation included 13 small red and black lines from Central America that performed well in a low soil fertility trial planted at the Isabela Substation in February 2007 (Dorcinvil, 2009) (Table 8). A split plot arrangement in a RCB experimental design with 5 replications was used. The whole plots were submitted two levels (0 and 25 kg ha^{-1}) of nitrogen fertilization as urea. The sub-plots were the 38 bean lines including the non-nodulating control (R-99 OAC Rico). The experimental units were single rows, 3 m in length, 0.6 m between rows and 30 seeds per row.

Table 7. Lines from the initial screening selected for additional evaluation based on nodulation and seed yield from the February 2007 planting.

Line	Seed type
Talamanca	Black
Arroyo Loro Negro	"
PR 0443-151	"
MH-43-2	"
ICTA Ostúa	"
PRF 9924-50N	"
DOR 500	"
Cardenal	Small red
DEHORO	"
Amadeus 77	"
VAX 3	"
RAB 655	"
SER 16	"
R-99 OAC Rico	White
Morales	"
OAC Rico	"
Verano	"
Beníquez	"
PR 0427-7	"
BAT 477	Cream
A 774	"
PR 0422-41	Andean
Redhawk	"
Salagnac 90A	"
PR 0422-39	"

Table 8. The promising 13 lines selected from the VIDAC black and small red seeds low fertility trials planted at Isabela, Puerto Rico during 2007 (Dorcinvil, 2009).

Line[1]	Seed type
RBF 11-36	Black
RBF 14-54	Black
MN 14059-7	Black
MEN 2207-44	Black
PR 0340-3-3-1	Red
RBF 11-29	Red
MER 2226-28	Red
IBC 301-182	Red
IBC 305-67	Red
IBC 309-23	Red
IBC 306-95	Red
IBC 308-83	Red
SEA 5	Pinto/cream

[1] Lines provided by Ronald Dorcinvil, 2007, Isabela Experimental Sub-Station, UPR-Mayaguez, PR.

All plots received 57 kg ha^{-1} of P_2O_5 and 54 kg ha^{-1} of K_2O at planting. All lines were inoculated as previously described for the February 2007 experiment. The field was maintained clean of weeds and irrigated when necessary to avoid stress.

On July 18-20, 2007 (42-44 DAP) nodulation scores were evaluated as described for the initial screening for BNF. The lines were harvested from 21 to 29 August, 2007. The harvest was conducted as was previously described for the experiments planted in February 2007.

Results were analyzed using ANOVA (Infostat, 2008) with a comparison of means using Fisher's LSD at 0.05 level of confidence. For an unbalanced data, a general and mixed linear model (Infostat, 2008) was used with a comparison of means using Fisher's LSD at 0.05 of confidence. A square root transformation was required to fulfill the normality and variance homogeneity assumptions for seed yield.

The third field evaluation for BNF was planted on October 5, 2007 during wet season at the same site. This was the third consecutive planting and a great amount of rain produced ideal conditions for root rot. The same lines from the June 2007 experiment were used (Tables 7 and 8). A split plot arrangement in a RCB experimental design with 5 replications was used. The whole plots were submitted two levels (0 and 25 kg ha^{-1}) of nitrogen fertilization in the form of urea. The sub-plots were the 38 bean lines. The same non-nodulating common bean line was used as control. The experimental units were single rows, 3 m in length, 0.6 m between rows and 30 seeds per row.

All plots received 57 kg ha^{-1} of P_2O_5 and 54 kg ha^{-1} of K_2O. All lines were inoculated as was described for the February and June 2007 experiments. The field was maintained clean of weeds and irrigated when necessary.

Due to the prevalence of root rot, the only parameter measured was plant vigor on December 10, 2007 (35 DAP). Plants were visually evaluated based on the CIAT 1-9 vegetative adaptation score where 1=excellent, 3=good, 5=intermediate, 7=poor, and 9=very poor (Van-Schoonhoven and Pastor-Corrales, 1987). Results were analyzed using ANOVA (Infostat, 2008) and a comparison of means using Fisher's LSD at 0.05 of level of confidence.

Evaluation of promising bean lines for BNF capacity at 25 kg ha^{-1} of N fertilization

The fourth field evaluation for BNF was planted on January 25, 2008 at the same site as the previous experiments. A soil chemical analysis was conducted before the last field experiment. Soil samples were taken at 0.15 m depth in each block. Soil samples were air dried and sieved (2 mm). They were analyzed at the MDS Harris Laboratory at Lincoln-Nebraska. NO_3^--N was extracted with 1M KCl solution and was colorimetric measured. Extractable P was determined using Bray-1 solution (0.025N HCl + 0.03N NH_4F) and quantified by colorimetric with Stannous chloride. The cations K, Mg, and Ca were extracted with Ammonium acetate and quantified using atomic absorption. Micronutrients (S, Zn, Mn, Cu, Fe, B) were measured with DTPA. The pH was determined with a 1:1 water/soil solution. Organic matter (OM) was quantified by ignition lost (MDS Harris, 2007).

The 13 most promising lines based on nodulation score, yield, and plant vigor from the June and October 2007 experiments were evaluated (Table 9). The non-nodulating line, R-99 OAC Rico was used as a control. The experimental design was a RCB with 5 replications. The experimental units were three rows, 3 m in length, 0.6 m between rows, 30 seeds per row, and 0.1 m between plants. A total of 90 seeds per plot were planted. This experiment was affected by drought, weeds and the insect pest *Liriomysa sativas* Blanchard, 1938 (leaf miner).

All plots were fertilized with 25, 50, and 50 kg ha^{-1} of N, P_2O_5, and K_2O, respectively. All lines were inoculated as described for previous experiments.

Table 9. Lines selected from June and October 2007 field experiments based on nodulation scores, seed yield, and plant vigor.

Line	Seed type
Arroyo Loro Negro	Black
PR 0443-151	"
MH-43-2	"
Cardenal	Small red
VAX 3	"
RAB 655	"
PR 0340-3-3-1	"
R-99 OAC Rico	White
Morales	"
OAC Rico	"
PR 0427-7	"
A 774	Cream
PR 0422-41	Red kidney
Salagnac 90A	Red mottled

The parameters measured in this experiment were: germination, nodulation score, shoot and root dry matter, yield components, and N content. Nodulation score, shoot dry weight (FAO, 2000), shoot N content, and shoot to root ratio were measured because they have been reported to have a high correlation with N fixation according to Graham et al. (2003).

Plant germination was evaluated due to poor emergence in many experimental units. The number of plants per experimental unit were counted on February 18, 2008 (24 DAP). The percentage of plant emergence was calculated.

The nodulation scores were measured as was previously described. Nodulation scores were evaluated at two different dates: March 13, 2008 (48 DAP) and April 1, 2008 (67 DAP).

The same plants collected for the nodulation were used to evaluate the shoot and root dry matter accumulation at 48 and 67 DAP. Roots were extracted carefully using a shovel, cut, marked and placed in a bucket of water to separate the bigger aggregates of soil from the root. Roots were water-soap cleaned to remove the soil excess. Shoots were marked and put in a paper bag. The samples were oven dried at 60℃ for 72 hours. The dried tissue was weighted and root to shoot ratios were calculated. At 67 DAP the same procedure was followed except that the pods were evaluated separately from the shoot and roots.

The components of seed yield were measured at 67 DAP using the same plants that were evaluated for nodulation scores and shoot and root dry matter accumulation. Pods of each plant were separated from the shoot and counted. Seeds from each pod were also counted and number of seeds per plant was calculated.

Shoot and seed samples were evaluated for N content using the Micro-Kjeldahl method (Bliss, 1993a; Bliss and Miller, 1988; Hardarson et al., 1993; Kipe-Nolt and Giller, 1993; Kipe-Nolt et al., 1993; Manrique et al., 1993; Moawad et al., 1998). Shoot (stem with leaves) and pods were oven dried at 60℃ for 72 h. Shoot and pods were ground, digested, distilled and titrated following the Micro-Kjeldahl method from the Laboratory Manual of Nutrition (Riquelme, 1998). The N percentage was multiplied by the dry weight to calculate kg ha^{-1} of N.

The plots were harvested during the period from April 22 to May 6, 2008 using the procedures described for previous experiments.

All lines were analyzed using ANOVA (Infostat, 2008) with a comparison of means using Fisher’s LSD at 0.05 level of confidence. In root dry matter at 48 DAP, root dry matter at 67 DAP, shoot dry matter at 48 DAP, shoot dry matter at

67 DAP, and in a hundred seed weight a square root transformation was required to fulfill the normality and variance homogeneity assumptions.

Results and Discussions

An initial screening of bean lines for BNF capacity was conducted in five field trials planted at the University of Puerto Rico Substation near Isabela, Puerto Rico. A soil sample was collected near the experimental site before the January 2008 field experiment. The chemical properties of the soil are presented in Table 10. The results indicate that the soil at the experimental site had low N content and moderate organic matter content (%). These are favorable soil conditions for screening beans for better adaptation to low soil N content. The population density of rhizobia in the soil was 5.7 x 10^2 cells g^{-1}. In a soil there can be a natural rhizobia densities ranging from $1x10^4$ to $1x10^7$ cells g^{-1}. If there is a substantial population of natural rhizobia, inoculation would not be so beneficial (Brockwell et al., 1995). Vincent (1975, from Velázquez-Báez, 1984) reported that abundant quantity of rhizobia in the soil ranges from 10^3-10^6 of rhizobia g^{-1} of soil. Meanwhile, Vargas et al. (2000) reported that inoculation had no effect on common bean nodulation and yield with a population of rhizobia of 7 x 10^2 cells g^{-1} soil.

Table 10. Chemical properties of the soil at the Isabela Experimental Substation.

pH	OM	NO_3^--N	P	K	Mg	Ca	CIC	S	Zn	Mn	Cu	Fe	B
	(%)	--(mg kg^{-1})---		------($cmol_c$ kg^{-1})-----				-----------------------(ppm)----------------------					
6.44	3.74	7	12	0.20	0.59	6.38	7.52	11.8	0.64	23.52	3.38	23.82	0.42
I [1]	I	L	I	I	L	I	I	---	I	H	H	H	---

[1] Soil fertility level: L=low, I=intermediate, and H=high (Sotomayor-Ramírez, 2006)

Initial field screening for nodulation and yield capacity

There were no significant differences in nodulation scores among the black bean lines. Only two of the 27 lines in the trial (7%), PRF 9924-50N (4.5) and SEN 3 (5.0) had intermediate nodulation scores (Table 11). PR 0443-151 (2,953 kg ha^{-1}) produced the greatest seed yield. The six lines (22%) that had seed yields greater than 1,500 kg ha^{-1} were MH-43-2 (1,825 kg ha^{-1}), Arroyo Loro Negro (1,692 kg ha^{-1}), Talamanca (1,692 kg ha^{-1}), ICTA Ostúa (1,675 kg ha^{-1}), and DOR 500 (1,642 kg ha^{-1}) (Table 12).

There were no significant differences in nodulation scores among the small red lines. Lines VAX 3 and Rojo de Seda had mean nodulation scores of 3.5 which were considered to be good. Cardenal, DICTA 113, DOR 364, DICTA 17, RAB 655, Rojo de Seda, Nueva Esperanza, CENTA Pupil, and SER 26 had mean nodulation scores ranging from 4 to 5 (Table 11). Nine of 27 lines (33%) had intermediate nodulation scores. VAX 3 combined a superior nodulation score (3.5) with a good seed yield (1,859 kg ha^{-1}). The lines with intermediate nodulation scores and mean seed yields > 1,500 kg ha^{-1} were Cardenal, RAB 655 and SER 16 (Tables 11 and 12).

There were significant differences in nodulation scores among the white lines. Four of the twelve lines (33%) had intermediate nodulation scores. PR 0427-7 had a mean nodulation score of 3.5. Morales, Beníquez, OAC Rico, and BelMiDak RMR 12 had intermediate nodulation scores ranging from 4 to 5 (Table 11). None of the white lines had mean seed yields greater than 1,500 kg ha^{-1}. PR 0427-7 and Morales had the best combination of nodulation scores (3.5 and 4.0, respectively) and seed yield (1,245 kg ha^{-1} and 1,228 kg ha^{-1}, respectively). Other lines with intermediate nodulation scores and good seed yield were: Beníquez (1,073 kg ha^{-1}) and OAC Rico (1,031 kg ha^{-1}).

There were no significant differences in nodulation scores among the pinto and cream lines. Only two of the 10 lines (20%) had an intermediate nodulation scores. USPT CBB-1 and BAT 477 had mean nodulation scores of 4.5 and 5.0, respectively (Table 11). BAT 477 had an intermediate nodulation score and a mean seed yield of 1,095 kg ha^{-1}. Only one of 10 lines (10%) had a seed yield greater than 1,500 kg ha^{-1}. A 774 had a mean seed yield of 2,809 kg ha^{-1}.

There were no significant differences in nodulation scores among the Andean bean lines. None of the lines had a mean nodulation score of less than 5. Redhawk had a mean nodulation score of 6.5 (Table 11). PR 0422-39 was the only line with a seed yield greater than 1,500 kg ha^{-1} (Table 12). Redhawk had poor nodulation and a relatively good seed yield (1,237 kg ha^{-1}).

Mean nodulation scores of the bean lines ranged from 3.5 to 9.0. A large amount of variability among common beans lines for N fixation has been previously reported (Bliss, 1993a; Hardarson et al., 1993; Herridge and Rose, 2000). The lines with mean nodulation scores of 3.5 were the small red lines VAX 3 and Rojo de Seda, and the white bean line PR 0427-7. The highest yielding bean lines was the black bean PR 0443-151 and the cream bean line A 774 with mean seed yields greater than 2,800 kg ha^{-1}. The small red and black bean lines had the best overall mean nodulation scores and seed yields (Tables 11 and 12).

Table 11. Nodulation scores of bean lines evaluated at flowering in trials planted at Isabela, Puerto Rico in February 2007.

Black lines	Nod[1]	Red lines	Nod[1]	White lines	Nod[1]	Pinto/Cream lines	Nod[1]	Andean lines	Nod[1]
PRF-9924-50N	4.5	VAX 3	3.5	PR0427-7	3.5	USPT CBB-1	4.5	Redhawk	6.5
SEN 3	5.0	SEDA	3.5	Morales	4.0	BAT 477	5.0	JB-178	7.0
B04316	5.5	Cardenal	4.0	Beniquez	4.0	ABCP-8	5.5	PR0422-39 (T-27)	7.5
Talamanca	6.0	DICTA 113	4.5	OAC Rico	4.5	A 774	6.0	PR9920-171	7.5
PR0443-151	6.0	DOR 364	4.5	BelMiDak RMR 12	5.0	Maverick	6.0	USCR 7	8.0
DOR 390	6.0	DICTA 17	4.5	BelMiDak RMR 10	5.5	USPT ANT-1	6.0	PR0422-28 (T-19)	8.0
Negro INIFAP	6.0	RAB 655	4.5	Vista	6.0	Carioca	6.5	PR0422-57 (T-41)	8.0
INTA Precoz	6.0	Rojo de Seda	5.0	Weihing	6.5	Othello	7.0	IJR	8.0
BCN-0202-94	6.0	Nueva Esperanza	5.0	Verano	6.5	Montrose	8.0	BRB-198	8.0
ICTA Ostua	6.5	CENTA Pupil	5.0	Matterhorn	7.0	P04205	8.0	Salagnac 90A	8.0
UCR 55	6.5	SER 26	5.0	Seahawk	7.5	Mean	6.3	NOD-125	8.0
Black Rhino	6.5	Macuzalito	5.5	R-99 OAC Rico	9.0	LSD (0.05)	NS	PC-50	8.0
SEN 21	6.5	Milineo	5.5	Mean	5.8	CV (%)	20.9	Cardinal	8.5
MH-43-2	6.5	SER 16	5.5	LSD (0.05)	2.1			PR0422-30 (T-21)	8.5
Rio Tibagi	7.0	SRS 6-6	6.0	CV (%)	18.8			PR0422-41 (T-28)	8.5
PR 0247-49	7.0	DEHORO	6.0					Rojo	8.5
B04554	7.0	Amadeus 77	6.0					PR0422-4 (T-3)	9.0
Aifi Wuriti	7.0	Carrizalito	6.0					PR0422-29 (T-20)	9.0
Jaguar	7.5	Bribri	6.0					Rosado	9.0
T-39	7.5	Tio Canela 75	7.0					Montcalm	9.0
Condor	7.5	VAX 6	7.5					Mean	8.1
B05055	7.5	SRS 56-3	7.5					LSD (0.05)	NS
DOR 500	8.0	Sedona	8.0					CV (%)	12.0
Midnight	8.0	PR9771-3-2	8.0						
B05040	8.0	Pamichial 1	8.5						
Porillo Sintetico	8.5	DICTA 122	8.5						
Arroyo Loro Negro	8.5	Merlot	9.0						
Mean	6.8	Mean	5.9						
LSD (0.05)	NS	LSD (0.05)	NS						
CV (%)	17.8	CV (%)	22.5						

[1] Nodulation score rated using the CIAT 1-9 scale: 1 = > 80 nodules plant^{-1}, 3 = 41-80 nodules plant^{-1}, 5 = 21-40 nodules plant^{-1}, 7 = 10-20 nodules plant^{-1} and 9 = < 10 nodules plant^{-1}

In this initial screening for BNF capacity, BAT 477 and DOR 364 had a mean nodulation scores of 5.0 and 4.5, respectively. Tang et al. (2001) found that BAT 477 and DOR 364 had similar nodulation and N_2 fixation in a glasshouse screening under low P. Meanwhile, Fernández-Toledo (1996) reported that DOR 364 had relative poor nodulation on both warm and cool season in the absence of N fertilization at Isabela. Carioca, ICTA Ostúa, Rio Tibagi, and Porrillo Sintético had nodulation scores ranging from poor to very poor. Araújo et al. (2000) reported that Rio Tibagi and Carioca had relatively a poor and intermediate nodulation respectively under two P levels in Brazil. In spite of their low nodules number, Rio Tibagi and Carioca were superior in grain yield (Araújo et al., 2000). Hardarson et al. (1993) evaluated the lines BAT 477, Rio Tibagi, Carioca, DOR 364, ICTA Ostúa, and Porrillo Sintético. In an experiment conducted in Austria, BAT 477 was ranked 6th among 29 lines in N derived from the atmosphere (%Ndfa), but was not a superior line based on fixed N (kg ha^{-1}) (Hardarson et al., 1993). In Guatemala, DOR 364 and ICTA Ostúa were not found to be superior in %Ndfa and fixed N (kg ha^{-1}) (Hardarson et al., 1993). Porrillo Sintético was not superior in %Ndfa and fixed N (kg ha^{-1}) in an experiment conducted in Mexico (Hardarson et al., 1993). In Brazil, Rio Tibagi had poor BNF capacity, but Carioca had good levels of %Ndfa and fixed N (kg ha^{-1}) (Hardarson et al., 1993).

The worst nodulation scores were found among the Andean lines with a mean nodulation score of 8.1 (≤ 10 nodules per plant). It could be that these lines had little or no infection after inoculation with *Rhizobium leguminosarum* biovar *phaseoli*. Nodari et al. (1993) found that Andean bean lines nodulated better with *Rhizobium tropici* strains. Most Andean lines had a determinate growth habit and were earlier in maturity that the Mesoamerican lines. Therefore, there was less of an opportunity for nodule formation on the Andean bean lines.

In general, intermediate to poor nodulation scores were observed in the initial screening for BNF. Singleton and Tavares (1986) found that in general inoculation increased legumes nodule number in a soil with less than 1×10^2 rhizobia per g. Meanwhile, a response was not found in soils with more than 20 rhizobia per g. An exception to this result was the snap bean cultivar 'Blue Lake', which had an increased number of nodules after inoculation in a soil with 1×10^4 rhizobia per g (Singleton and Tavares, 1986). Nodulation may have been affected by the amount of N fertilizer (50 kg ha^{-1}) applied to the field trials. As little as 20 to 50 kg N ha^{-1} has been reported to suppress nodule formation and activity in common bean (Da Silva et al., 1993; Dean and Clark, 1980; Saito et al., 1984; Vargas et al., 2000). Also, the rhizobia inoculated to the soil can be suppressed by the native *Rhizobium* (Aguilar et al., 2001). It has been found that competition of native species deserves special attention because it can affect N fixation capacity of bean cultivars (Vásquez-Arroyo et al., 1998). Also, the infection and nodulation of some strains of *Rhizobium* can be blocked by others (Martínez-Romero et al., 1998) causing a reduction in N fixation. Singleton and Tavares (1986) found that the inoculated *Rhizobium* can compete with indigenous strains, but this does not necessarily improve BNF. In two pea cultivars, Winarno and Lie (1979) reported that nodulation was controlled by a non-nodulating *Rhizobium* strain. Meanwhile, Piha and Munns (1987) found that no competition by the native rhizobia affected the symbiosis in common bean.

PR 0443-151, MH-43-2, VAX 3, and A 774 produced mean seed yields greater than 1,800 kg ha^{-1} with an application of 50 kg N ha^{-1}. In Lajas, Puerto Rico Velázquez-Báez (1984) reported a mean seed yield of 1,863 kg ha^{-1} using a 100 kg N ha^{-1} of fertilizer application rate. In two field experiments at Isabela, Puerto Rico, Cordero-García (1979) reported a mean seed yield of 2,128 and 2,488 kg ha^{-1} with an application of 112 kg N ha^{-1}, respectively. Under no N application rate, Cordero-García (1979) reported a mean seed yield of 1,711 and 1,737 kg ha^{-1}, respectively. At the second experiment Cordero-García (1979) reported a mean yield of 2,077 kg ha^{-1} with 56 kg ha^{-1} of N. This difference may be due to the greater seed yield potential and N use efficiency of more recently developed bean breeding lines.

Table 12. Common bean seed yield performance under low soil fertility trials at Isabela, PR in April, 2007.

Black lines	Yield (kg/ha)	Red lines	Yield (kg/ha)	White lines	Yield (kg/ha)	Pinto cream lines	Yield (kg/ha)	Andean lines	Yield (kg/ha)
PR0443-151	2953	VAX 3	1859	PR0427-7	1245	A 774	2809	PR0422-39 (T-27)	1667
MH-43-2	1825	RAB 655	1723	Morales	1228	Carioca	1300	PR0422-41 (T-28)	1468
Arroyo Loro Negro	1692	SER 16	1528	Verano	1192	BAT 477	1095	Salagnac 90A	1284
Talamanca	1692	Cardenal	1509	Beniquez	1073	USPT ANT-1	900	Redhawk	1237
ICTA Ostua	1675	SRS 56-3	1417	OAC Rico	1031	Othello	881	PR0422-29 (T-20)	1214
DOR 500	1642	Tio Canela 75	1411	Vista	914	Montrose	809	PR0422-4 (T-3)	1192
DOR 390	1364	DEHORO	1406	BelMiDak RMR 10	809	Maverick	750	PR9920-171	1089
Aifi Wuriti	1334	DOR 364	1403	Matterhorn	753	ABCP-8	745	NOD-125 (no-nod)	1067
UCR 55	1253	Macuzalito	1361	Weihing	725	USPT CBB-1	700	PR0422-57 (T-41)	1025
PRF-9924-50N	1203	Amadeus 77	1331	BelMiDak RMR 12	711	P04205	589	Rojo	1025
Porillo Sintetico	1170	DICTA 122	1273	Seahawk	692	Mean	1058	Montcalm	1017
BCN-0202-94	1150	CENTA Pupil	1266	R-99 OAC Rico	658	CV (%)	388	PC-50	981
INTA Precoz	1147	Sedona	1220	Mean	919	LSD (0.05)	31.6	IJR	978
Rio Tibagi	1117	DICTA 113	1198	CV (%)	278			PR0422-30 (T-21)	972
Negro INIFAP	1103	Milineo	1161	LSD (0.05)	26.2			PR0422-28 (T-19)	914
Condor	986	Rojo de Seda	1142					Rosado	781
SEN 3	975	Nueva Esperanza	1134					JB-178	706
B04554	959	SER 26	1125					USCR 7	705
SEN 21	936	Carrizalito	1100					BRB-198	581
B04316	859	Pamichial 1	1034					Cardinal	431
T-39	828	VAX 6	986					Mean	1017
Midnight	828	SEDA	908					CV (%)	371
B05040	781	Bribri	870					LSD (0.05)	31.7
B05055	759	DICTA 17	867						
Jaguar	752	SRS 6-6	860						
PR 0247-49	503	PR9771-3-2	772						
Black Rhino	380	Merlot	383						
Mean	1180	Mean	1194						
CV (%)	351	CV (%)	402						
LSD (0.05)	26.1	LSD (0.05)	29.4						

There was a significant (*p*<0.05) and negative correlation (r=-0.42) between mean seed yield and nodulation score among the small red lines. The same association was found for white lines with a significance of *p*<0.05 and r=-0.71. Some of the highest yielding small red and white lines had good to intermediate levels of nodulation. No significant correlations were observed between seed yield and nodulation scores for the black, pinto/cream and Andean seed types.

Evaluation of promising bean lines for nodulation and yield capacity at low levels (0 and 25 kg ha^{-1}) of N fertilization

The second field experiment evaluated the performance of 38 bean lines at two N fertilization levels (0 and 25 kg ha^{-1}) (Tables 7 and 8).

There was no significant interaction between lines and fertility on nodulation score (Table 13). There were significant differences (*p*<0.05) among bean lines in mean nodulation scores (Table 13). Four of forty (10%) lines, Salagnac 90A, Arroyo Loro Negro, PR 0427-7, and MH-43-2 had mean nodulation scores ranging from 2.7 to 3.7 (Table 13). The moderate level of fertilization in the preliminary trials (50 kg N ha^{-1}) may have suppressed the nodulation of Salagnac 90A and Arroyo Loro Negro. On the other hand, PR 0427-7 had among the best nodulation scores in the preliminary and second field screenings for BNF. This line appears to be a good nodulator under different N levels (0, 25 and 50 kg N ha^{-1}) (Tables 11 and 15).

A large amount of variation among lines in mean nodulation scores (2.7-8.3) was observed for the second field screening for BNF (Table 15). Variability among common bean lines for N fixation and its components including nodulation score have been reported (Bliss, 1993a; Hardarson et al., 1993; Herridge and Rose, 2000).

Ten of the forty (25%) lines had an intermediate mean nodulation scores ranging from 4.0 to 5.0 (MER 2226-28, RBF 11-29, ICTA Ostúa, RBF 14-54, BAT 477, Talamanca, PR 0340-3-3-1, RBF 11-36, RAB 655, and MER 2226-24). RAB 655 and BAT 477 had intermediate mean nodulation scores in both the preliminary and second field screening for BNF (February and June 2007 planting dates) (Tables 11 and 15). Talamanca had a better nodulation score during the summer. Fernández-Toledo (1996) reported Talamanca having a better nodulation during the warm season in comparison to the cool season when no N fertilizer was applied at Isabela. Non-nodulating line R-99 OAC Rico had very poor nodulation (about 10 nodules plant^{-1}) (nodulation score of 8.3) (Table 15). Before releasing this line, Park and Buttery (2006) found some nodulation by nine of eighteen (50%) different *Rhizobium leguminosarum* bv. *phaseoli* strains. They concluded that an interaction among some strains and the non-nodulating line was possible (Park and Buttery, 2006).

There were significant differences in the nodulation scores at different levels of N fertilization (Table 13). Lower nodulation scores (i.e. more nodulation) were observed at the low N level (0 kg ha^{-1}) with an intermediate level of nodulation (nodulation score of 4.3, about 40 nodules per plant). When the level of N fertilization increased from 0 to 25 kg ha^{-1}, mean nodulation scores increased from 4.3 to 6.4 (Table 14). Some authors have reported that increased N in the soil tends to depress nodulation (Catano-Catano, 1990; Mangual-Crespo et al., 1987;

Müller et al., 1993; Vargas et al., 2000). Catano-Catano (1990) found that the application of N fertilizer reduced the number and weight of nodules; however, it increased the yield and total N in the seed.

Table 13. Analysis of variance of nodulation scores in the June 2007 experiment.

Source of Variation	df	df Error	F-value	*p*-value
Fertility	1	2	79.33	0.0124
Identity	37	147	2.56	< 0.0001
Fertility x Identity	37	147	1.14	0.2904

Table 14. Mean of nodulation scores at two N fertility levels (0 and 25 kg ha^{-1}) in the June 2007 experiment.

N (kg ha^{-1})	Nodulation score
0	4.3
25	6.5
Mean	5.4
LSD (0.05)	1.0
CV (%)	34.0

Table 15. Mean of nodulation scores of common bean lines in the June 2007 experiment.

Identity	Mean nodulation score^1
Salagnac 90A	2.7
Arroyo Loro Negro	2.8
PR0427-7	3.5
MH-43-2	3.7
MER 2226-28	4.0
RBF 11-29	4.2
ICTA Ostúa	4.5
RBF 14-54	4.7
BAT 477	4.8
PR 0340-3-3-1	4.8
RAB 655	4.8

Table 15. Continued.

Identity	Mean nodulation score[1]
Talamanca	4.8
RBF 11-36	4.8
Morales	5.2
DOR 500	5.2
Cardenal	5.2
Amadeus 77	5.2
A774	5.3
PR0443-151	5.3
PRF 9924-50N	5.5
PR0422-41	5.5
VAX 3	5.7
OAC Rico	5.7
Verano	5.7
IBC 301-182	5.7
IBC 305-67	5.8
Beníquez	5.8
MEN 2207-44	5.8
IBC 309-23	6.0
IBC 306-95	6.2
DEHORO	6.2
Redhawk	6.2
IBC 308-83	6.5
SER 16	6.7
MN 14059-7	6.8
SEA 5	7.3
PR0422-39	7.7
R-99 OAC Rico	8.3
Mean	5.4
LSD (0.05) Except for line Redhawk	2.1
CV (%)	34.0

[1] Nodulation rated using the CIAT 1-9 scale: 1 = > 80 nodules plant^{-1}, 3 = 41-80 nodules plant^{-1}, 5 = 21-40 nodules plant^{-1}, 7 = 10-20 nodules plant^{-1} and 9 = < 10 nodules plant^{-1}.

A square root transformation of seed yield was necessary to fulfill the normality and variance homogeneity assumptions. In the analysis of variance for the second evaluation for BNF, there was no significant interaction between lines and fertility on seed yield (square root transformed means) (Table 16). There were significant differences in the seed yield (square root transformed means) at different levels of N fertilization (Table 16). Mean seed yield at 25 kg N ha^{-1} (788 kg ha^{-1}) was 1.84 times greater than the 0 kg N ha^{-1} (428 kg ha^{-1}) application rate (Table 17). Catano-Catano (1990) found that the application of N fertilizer increased the yield. There were significant differences ($p<0.05$) among bean lines in mean seed yield (square root transformed means) (Table 16). Lines with the highest mean seed yield were VAX 3, PR 0443-151, A 774, and PR 0340-3-3-1. These lines had mean seed yields greater than 1,000 kg ha^{-1} (Table 18). These lines were better adapted to low N conditions. In 20 years of selection for low fertility soil tolerant common bean lines, Singh et al. (2003) reported line A 774 having the highest seed yield (948 kg ha^{-1}) under low soil fertility. Fernández-Toledo (1996) also identified common bean lines at the Isabela Substation that had mean seed yields greater than 1,000 kg ha^{-1} without N fertilizer and nodulation with native rhizobia. These results contrasted with some investigations that found a limited amount of N (25 kg N ha^{-1}) produced seed yields of greater than 1,500 kg ha^{-1} (Kipe-Nolt et al., 1993; Mangual-Crespo et al., 1987). VAX 3, A 774, PR 0443-151, and PR 0340-3-3-1 had an intermediate amount of nodulation (about 30 nodules per plant). This suggests that these lines were able to fix a reasonable amount of N_2 with less number of nodules or that these lines were more efficient in the use of N. Depending solely on BNF, Fernández-Toledo (1996) identified that bean lines that were efficient in the use and acquisition of N permitted to produce seed yields greater than 1,000 kg ha^{-1} in low N conditions.

Table 16. Analysis of variance of seed yield in the June 2007 experiment.

Square root transformation				
Source of Variation	df	df Error	F-value	*p*-value
Fertility	1	4	9.30	0.0380
Identity	37	266	15.88	< 0.0001
Fertility x Identity	37	266	0.77	0.8318
Plant number	1	266	186.73	< 0.0001

Table 17. Adjusted Means (LSMeans) of seed yield at two N fertility levels (0 and 25 kg ha^{-1}) in the June 2007 experiment.

Square root transformation			Reversed transformation	
N (kg ha^{-1})	Seed yield	SE	N (kg ha^{-1})	Seed yield (kg ha^{-1})
0	20.68	1.54	0	427.66
25	28.08	1.53	25	788.49
Mean	24.38			
LSD (0.05)	6.03			
CV (%)	19.69			

Table 18. Adjusted Means (LSMeans) of seed yield of common bean lines in the June 2007 experiment.

Square root transformation			Reversed transformation	
Identity	Seed yield	SE	Identity	Seed yield (kg ha^{-1})
VAX 3	34.48	1.98	VAX 3	1188.87
PR0443-151	33.51	1.96	PR0443-151	1122.92
A774	32.49	2.01	A774	1055.60
PR 0340-3-3-1	31.76	2.08	PR 0340-3-3-1	1008.70
RBF 11-36	28.51	1.93	RBF 11-36	812.82
Arroyo Loro Negro	27.60	1.95	Arroyo Loro Negro	761.76
MN 14059-7	27.36	1.93	MN 14059-7	748.57
RAB 655	27.35	1.97	RAB 655	748.02
IBC 309-23	27.16	1.93	IBC 309-23	737.67
RBF 11-29	26.88	2.02	RBF 11-29	722.53
PRF 9924-50N	26.57	2.01	PRF 9924-50N	705.96
ICTA Ostúa	26.24	1.93	ICTA Ostúa	688.54
MEN 2207-44	25.95	1.93	MEN 2207-44	673.40
MH-43-2	25.34	1.94	MH-43-2	642.12
SER 16	25.18	2.01	SER 16	634.03
DEHORO	25.11	1.93	DEHORO	630.51
Verano	24.31	1.95	Verano	590.98

Table 18. Continued.

Square root transformation			Reversed transformation	
Identity	Seed yield	SE	Identity	Seed yield (kg ha^{-1})
Amadeus 77	23.78	1.93	Amadeus 77	565.49
Cardenal	23.70	2.41	Cardenal	561.69
Morales	23.38	1.95	Morales	546.62
IBC 301-182	23.26	2.02	IBC 301-182	541.03
IBC 306-95	23.00	2.13	IBC 306-95	529.00
Talamanca	22.47	1.95	Talamanca	504.90
Salagnac 90A	22.31	1.94	Salagnac 90A	497.74
DOR 500	22.22	1.93	DOR 500	493.73
IBC 305-67	22.12	2.01	IBC 305-67	489.29
PR0422-41	21.86	2.05	PR0422-41	477.86
MER 2226-28	21.56	1.93	MER 2226-28	464.83
PR0422-39	21.52	2.25	PR0422-39	463.11
IBC 308-83	21.50	2.03	IBC 308-83	462.25
BAT 477	21.36	1.95	BAT 477	456.25
RBF 14-54	21.00	2.01	RBF 14-54	441.00
R-99 OAC Rico	20.66	2.13	R-99 OAC Rico	426.84
PR0427-7	20.33	2.35	PR0427-7	413.31
SEA 5	19.51	2.03	SEA 5	380.64
Beníquez	18.70	1.93	Beníquez	349.69
Redhawk	18.35	2.42	Redhawk	336.72
OAC Rico	17.94	2.01	OAC Rico	321.84
Mean	24.38			
Approx. LSD (0.05)[1]	5.61	Using SAV (2.03)		
CV (%)	19.69			

[1] Approximated LSD calculated using the Square root of the Average Variances (SAV) (Casanoves et al., 2005).

There was no significant correlation between mean nodulation scores and seed yield. Eleven lines had a hundred seed weight more than 25 g (medium seed size) (Table 19) (Van-Schoonhoven and Pastor-Corrales, 1987). The remaining lines had a small seed size (<25 g) (Table 19).

Table 19. Mean of a hundred seed weight of common bean lines in the June 2007 experiment.

Identity	Hundred seed weight (g)
Redhawk	29.3
PR 0422-41	28.8
VAX 3	27.7
RAB 655	27.5
MN 14059-7	25.8
PR 0422-39	25.7
A 774	25.7
Amadeus 77	25.7
SER 16	25.6
MH-43-2	25.3
RBF 11-29	25.3
IBC 306-95	24.8
Cardenal	24.5
IBC 309-23	24.3
RBF 14-54	24.1
DEHORO	23.8
Salagnac 90A	23.8
MER 2226-28	22.7
RBF 11-36	22.2
SEA 5	22.1
PR 0340-3-3-1	21.8
Verano	21.3
IBC 301-182	21.2
PRF 9924-50N	21.0
IBC 305-67	21.0
MEN 2207-44	21.0
IBC 308-83	20.8
BAT 477	20.7
ICTA Ostúa	20.5
Arroyo Loro Negro	20.5
Talamanca	19.8
Morales	19.7
PR 0427-7	18.5
DOR 500	18.3
Beníquez	18.2
R-99 OAC Rico	16.3
PR 0443-151	16.2
OAC Rico	15.8
Mean	22.2
LSD (0.05)	2.1
CV (%)	8.0

The field experiment planted in October 2007 was affected by root rot. This was the third consecutive planting in the same site. During three weeks after planting, an excessive amount of rainfall (122 mm) (Table 1) created conditions favorable for root rot development. Miklas et al. (2006) reported that a region with low fertility soils, limited crop rotation and multiple season production promotes root rot infestations. The plant vigor scores provided an estimate of the damage caused by root rot. This root rot could have been caused by one or a combination of the following pathogens (*Fusarium solani*, *Macrophomina phaseolina*, *Pythium* sp., *Rhizoctonia* sp.). There was a significant interaction ($p<0.05$) between lines and rates of N fertilization (Table 20). The lines with the best plant vigor scores in the 0 N plots were RAB 655 (3.67), PR 0443-151 (3.67), and VAX 3 (4.33) (Table 21). The lines with the best plant vigor scores in the 25 kg N ha^{-1} plots were RAB 655 (3.00), VAX 3 (4.00), A774 (4.00), and Arroyo Loro Negro (4.00) (Table 21). RAB 655 had the best overall plant vigor scores in the two N levels (Table 21). This line also had an intermediate level of nodulation in the first two experiments (4.5 and 4.8, respectively). Another line which had a similar response at two N levels was VAX 3. Lines with the best plant vigor score are presented in Figure 1. VAX 3 and PR 0443-151 are considered to have useful levels of root rot resistance. A 774 and RAB 655 have tolerance to low fertility soils.

Table 20. Analysis of variance of plant vigor score in the October 2007 experiment.

Source of Variation	df	MS	*p*-value
Identity	38	12.94	<0.0001
Fertility	1	19.18	0.0356
Identity*Fertility	38	1.47	0.0247
Block	2	12.04	0.0566
Error	152	0.92	

Table 21. Mean of plant vigor scores at two N levels (0 and 25 kg N ha^{-1}) in common bean lines in the October 2007 experiment.

Identity	Mean plant vigor scores^{1}		
	0 kg N ha^{-1}	25 kg N ha^{-1}	Mean
RAB 655	3.7	3.0	3.3
VAX 3	4.3	4.0	4.2
A774	5.0	4.0	4.5
Arroyo Loro Negro	5.3	4.0	4.7
PR0443-151	3.7	4.7	4.2
Talamanca	5.3	5.3	5.3
MH-43-2	6.7	5.3	6.0
MER 2226-28	7.7	5.3	6.5
Salagnac 90A	8.7	5.3	7.0
PR 0340-3-3-1	6.0	5.7	5.8
IBC 305-67	6.3	5.7	6.0
MER 2226-24	7.0	5.7	6.3
DOR 500	7.7	5.7	6.7
ICTA Ostúa	7.3	6.0	6.7
RBF 11-29	6.0	6.3	6.2
BAT 477	8.0	6.3	7.2
Cardenal	8.0	6.3	7.2
PR0422-39	7.7	6.7	7.2
IBC 308-83	9.0	6.7	7.8
SER 16	6.3	7.0	6.7
PRF 9924-50N	7.7	7.0	7.3
Redhawk	8.0	7.0	7.5
DEHORO	8.0	7.7	7.8
PR0422-41	8.0	7.7	7.8
PR0427-7	8.7	7.7	8.2
RBF 11-36	7.0	8.0	7.5
MN 14059-7	7.7	8.0	7.8
IBC 306-95	8.3	8.0	8.2

Table 21. Continued.

Identity	Mean plant vigor scores[1]		
	0 kg N ha^{-1}	25 kg N ha^{-1}	Mean
RBF 14-54	8.3	8.0	8.2
IBC 301-182	8.3	8.3	8.3
OAC Rico	9.0	8.3	8.7
Amadeus 77	8.0	8.7	8.3
Morales	8.0	8.7	8.3
SEA 5	8.3	8.7	8.5
IBC 309-23	8.7	8.7	8.7
Beníquez	9.0	8.7	8.8
R-99 OAC Rico	9.0	8.7	8.8
Verano	9.0	8.7	8.8
MEN 2207-44	8.0	9.0	8.5
Mean	7.4	6.8	
LSD (0.05)	1.6		
CV (%)	13.58		

[1] Plant vigor based on a 1-9 scale: 1 = Excellent, 3 = Good, 5 = Intermediate, 7 = Poor and 9 = Very poor.

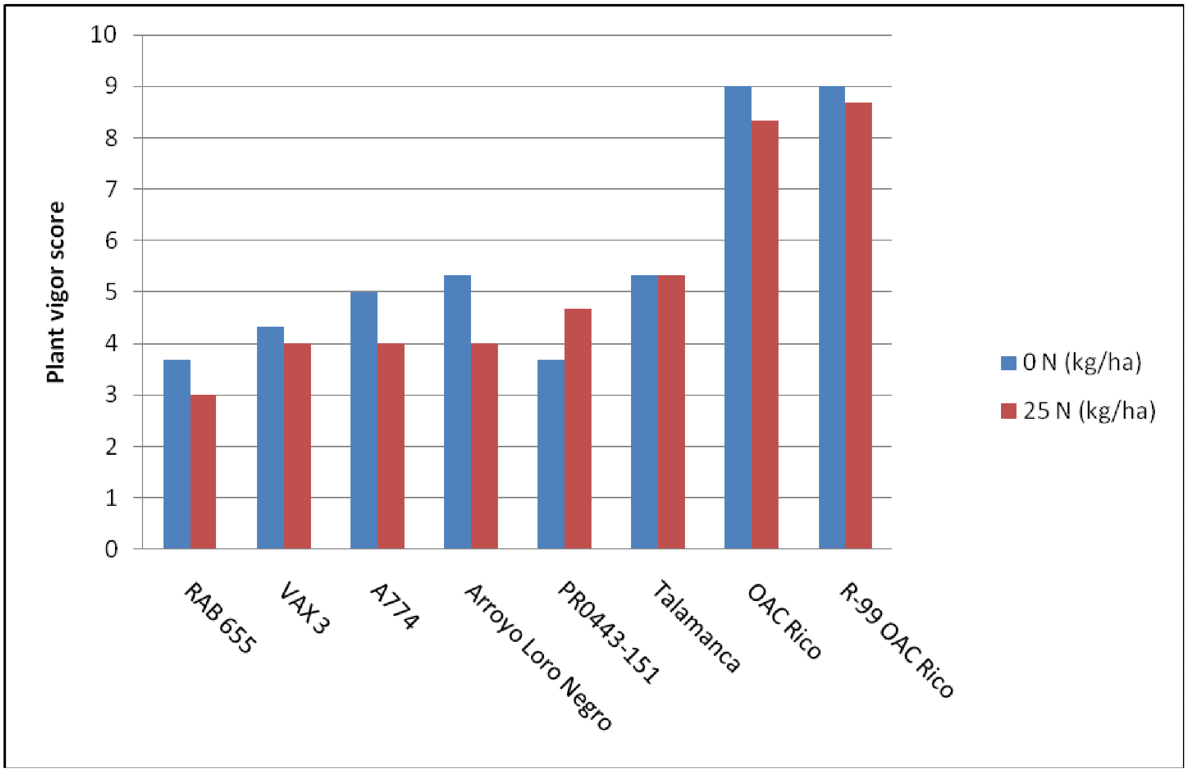


Figure 1. Best common bean lines based on plant vigor visual score for root rot in the October 2007 experiment.

There was a significant ($p < 0.05$) positive correlation (r=0.38) between the June 2007 nodulation scores and the plant vigor visual score for root rot of the October 2007 field experiment. Root rot severity was associated with better nodulation scores. Brockwell et al. (1995) observed that legume nodulation can be negatively affected by root rot (Brockwell et al., 1995). Biotic or abiotic factors that affect plant vigor and photosynthesis will have an indirect effect on BNF (Brockwell et al., 1995; Zahran, 1999).

In a glasshouse experiment, Muthomi et al. (2007) found that inoculation of bean plants with *Fusarium oxysporum* and *Macrophomina phaseolina* had no effect on nodulation. However, they found a reduction in nodule number when fungicide alone and combined with a pathogen was applied on seeds. They recommended the use of both fungicide and inoculation to improve nodulation and controlling root rot for legumes. Inoculation increased nodulation and shoot dry matter. Fungicide alone helped to reduce root rot, but can also diminish nodulation (Muthomi et al., 2007).

In a greenhouse experiment, Khalequzzaman and Hossain (2007) treated bushbean seeds with *Rhizobium* strains and biofertilizers to control *Fusarium solani* foot and root rot disease. The lowest seed rot, foot rot, root rot and greater germination and plant stand was found with BINAR P36 *Rhizobium* strain and BINA biofertilizer. They concluded that BINAR P36 can be used for reducing foot and root rot and increasing bushbean yield (Khalequzzaman and Hossain, 2007).

In a laboratory experiment, Sharif et al. (2003) found suppression from legume and non-legume pathogens by the Thal-8, a *Rhizobium* sp. strain. This effect was caused by organic acids, antibiotics and toxic metabolites produced by rhizobia. They recommended the use of a mixture of different *Rhizobium* strains to improve root rot control (Sharif et al., 2003).

Ehteshamul-Haque (1994) reported an inhibition zone created by *Rhizobium* sp. (KUMH 770) and *Bradyrhizobium* sp. (KUMH 560). He also reported that the strain *Rhizobium meliloti* (KUMH 653) suppressed *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium oxysporum*. *Bradyrhizobium japonicum* (KUMH 569 and KUMH 566) suppressed *Macrophomina phaseolina* and *Rhizoctonia solani*. *R. meliloti* (KUMH 139 and KUMH 555), *B. japonicum* (KUMH 569) and *R. leguminosarum* (KUMH 551) applied to seed and soil limited *M. phaseolina*, *R. solani* and *Fusarium* spp. on legumes (soybean and mungbean, *Vigna radiata* L.) and non-legumes in the field. The strain that controlled better the root rot was *B. japonicum* strain TAL-102. He recommended the use of a combination of *Rhizobium* with *Trichoderma* spp., *Paecilomyces lilacinus*, *Gliocladium virens* and *Stachybotrys atra* as a practical management tool for root rot control. Combinations of *B. japonicum* with *Trichoderma hamatum* increased soybean seed yield. Rhizobia mixed with *P. lilacinus* enhanced mungbean nodulation.

Macrophomina phaseolina and *Fusarium* sp. were found in the soil of the experimental site (Appendixes 1 and 2). In Puerto Rico, Fernández-Toledo (1996) reported the presence of *M. phaseolina* in the second year of planting at the Isabela Experimental Substation. Cordero-García (1979) reported problems at the Isabela Substation in emergence and first stage development of common bean caused by *Macrophomina*, *Rhizoctonia*, *Pythium*, and *Fusarium*, during the rainy season. At the Lajas Experimental Substation, Guadalupe-Luna (1977) observed that *Macrophomina* sp., *Rhizoctonia* sp., *Phytium* sp., and *Fusarium* sp. affected bean growth and development.

Evaluation of promising bean lines for BNF capacity at 25 kg ha^{-1} of N fertilization

The field experiment planted in January 2008 was affected by drought, weeds and *Liriomysa sativas* insect (leaf miner). Water needs to be sufficient to permit roots to develop and increase the infection area available for *Rhizobium* (Santalla et al., 2001). Bean producers in developing countries often encounter drought, disease and pest pressure. The parameters measured in this experiment were: germination, nodulation score, shoot and root dry matter, yield components, and N content. There was a significant difference (p<0.05) in germination among bean lines (Table 22). Salagnac 90A had the highest percent germination (94%) (Table 23). Only 3 lines (Salagnac 90A, PR 0443-151 and Morales) had germination percentages of more than 85% (Table 23). The remaining lines in the trial had germination of 60% or less (Table 23).

Table 22. Analysis of variance of germination percentage in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	3354.29	<0.0001
Block	4	96.66	0.0056
Error	52	23.41	

Table 23. Mean of germination percentage in the January 2008 experiment.

Line	Germination (%)
Salagnac 90A	94.5
PR 0443-151	88.2
Morales	86.9
Arroyo Loro Negro	62.0
PR 0427-7	52.9
A 774	52.2
VAX 3	51.3
RAB 655	51.1
PR 0340-3-3-1	48.7
R-99 OAC Rico	39.3
OAC Rico	38.7
Cardenal	36.7
MH 43-2	10.9
PR 0422-41	7.1
Mean	51.5
LSD (0.05)	6.1
CV (%)	9.4

At 48 DAP, there was no significant difference among lines in nodulation scores (Table 24). This is comparable to an experiment in Lajas by Vázquez-Báez (1984) where there was no difference in plant nodule number at 39 DAP. Drought and low soil fertility are abiotic factors that can affect nodulation. Peña-Cabriales and Castellanos (1993) found a decrease in nodule number in two common bean cultivars when exposed to water stress during the vegetative stage of development. De Oliviera et al. (1998) reported that nodule number was affected by fertilization under controlled conditions. However, there was a significant difference (p<0.05) among lines in nodulation scores at 67 DAP (Table 25). Vázquez-Báez (1984) found significant differences in nodulation number at 56 DAP. The line with the best mean nodulation score at 67 DAP was OAC Rico

(Table 26). OAC Rico and PR 0427-7 had an acceptable amount of nodulation in the previous experiments.

At 67 DAP, the lines with the best nodulation scores were OAC Rico and PR 0427-7 (Table 26). OAC Rico and PR 0427-7 averaged more than 40 nodules per plant. In general, the white bean lines OAC Rico and PR 0427-7 had the best overall nodulation scores (Table 27). Kipe-Nolt and Giller (1993) found that in general lines with higher number and weight of nodule fixed a greater amount of N. Meanwhile, Hansen et al. (1992) reported a supernodulating mutant (R32BS15) capable of producing a higher number of nodules in the presence of nitrate in the soil. The BNF capacity of this mutant was affected negatively by the presence of nitrate (Hansen et al., 1992).

Comparing OAC Rico with its counterpart, R-99 OAC Rico, which had very poor nodulation (a mean of less than 10 nodules per plant), Park and Buttery (2006) reported that OAC Rico produced up to five times more nodules than R-99 OAC Rico. Hansen et al. (1992) found that the supernodulating mutant R32BS15 produced up to 15 times more nodules than the wild type cultivar OAC Rico at the highest nitrate rate (6 mol m^{-3}).

Table 24. Analysis of variance of nodulation score at 48 DAP in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Identity	13	1.20	0.8425
Block	2	2.76	0.2784
Error	26	2.05	

Table 25. Analysis of variance of nodulation score at 67 DAP in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Identity	13	10.95	0.0006
Block	2	8.15	0.0514
Error	26	2.44	

Table 26. Mean of nodulation score at 48 and 67 DAP in the January 2008 experiment.

Line	Mean nodulation scores[1]	
	48 DAP	67 DAP
OAC Rico	6.8	1.7
PR 0427-7	6.8	4.7
Morales	7.7	5.0
Cardenal	8.5	5.0
PR 0443-151	7.5	6.0
VAX 3	7.2	6.5
RAB 655	7.2	6.7
PR 0422-41	7.7	6.8
Salagnac 90A	7.2	7.0
PR 0340-3-3-1	8.0	7.0
MH 43-2	7.5	7.3
Arroyo Loro Negro	6.8	8.0
A 774	7.5	9.0
R-99 OAC Rico	9.0	9.0
Mean	7.5	6.4
LSD (0.05)	NS	2.6
CV (%)	19.0	24.4

[1] Nodulation rated using the CIAT 1-9 scale: 1 = > 80 nodules plant^{-1}, 3 = 41-80 nodules plant^{-1}, 5 = 21-40 nodules plant^{-1}, 7 = 10-20 nodules plant^{-1} and 9 = < 10 nodules plant^{-1}.

Table 27. Mean of nodulation score at Isabela in the February 2007, June 2007, and January 2008 experiments.

Line	Mean nodulation score[1]			
	February 2007 45 DAP	June 2007 42-44 DAP	January 2008 48 DAP	January 2008 67 DAP
PR 0427-7	3.5	3.5	6.8	4.7
OAC Rico	4.5	5.7	6.8	1.7
Mean of the entire experiment	5.8	5.4	7.5	6.4

[1] Nodulation rated using the CIAT 1-9 scale: 1 = > 80 nodules plant^{-1}, 3 = 41-80 nodules plant^{-1}, 5 = 21-40 nodules plant^{-1}, 7 = 10-20 nodules plant^{-1} and 9 = < 10 nodules plant^{-1}.

The lines that had the best overall mean nodulation scores were the white beans PR 0427-7 and OAC Rico (Table 27). PR 0427-7 had the best mean nodulation score in the February 2007 field experiment, among the best nodulation scores in the June 2007 and January 2008 field experiments. Ranking of mean nodulation scores is an efficient method to identify superior lines (Henson, 1993).

In general, Bliss (1993a), Hardarson et al. (1993), and Santalla et al. (2001) reported that the indeterminate and climbing cultivars have the capacity of nodulating and fixing more N than determinate cultivars. There was a large amount of variation in nodulation scores at 67 DAP (1.67-9.00). A high degree of variability among lines for N fixation and its components has been reported for bean (Bliss, 1993a; Catano-Catano, 1990; Hardarson et al., 1993; Herridge and Rose, 2000).

There was no significant correlation between the plant vigor score for root rot at 0 kg N ha^{-1} of the October 2007 experiment and the nodulation scores at 48 and 67 DAP of the January 2008 field experiment. There was no significant correlation between the plant vigor score for root rot at 25 kg N ha^{-1} of the October 2007 experiment and the nodulation scores at 48 and 67 DAP of the January 2008 field experiment. There was no significant correlation between the plant vigor score for root rot (using general mean including the two levels of N fertilizer) of the October 2007 experiment and the nodulation scores at 48 and 67 DAP of the January 2008 field experiment.

A square root transformation of the root dry matter data was necessary to fulfill the normality and variance homogeneity assumptions. There was a significant difference among bean lines (p<0.05) in root dry matter (square root transformed) at 48 DAP and 67 DAP (Tables 28 and 29). The lines with the greatest root dry matter at both sampling dates were MH 43-2, PR 0340-3-3-1, and VAX 3. MH 43-2 (0.510 and 1.19 g plant^{-1}), PR 0340-3-3-1 (0.465 and 1.06 g plant^{-1}), and VAX 3 (0.412 and 1.04 g plant^{-1}) had higher root dry matter at 48 and 67 DAP, respectively (Tables 30 and 31).

Table 28. Analysis of variance of root dry matter at 48 DAP in the January 2008 experiment.

Square root transformation			
Source of Variation	df	MS	*p*-value
Identity	13	0.015	0.0020
Block	2	0.002	0.5601
Error	22	0.004	

Table 29. Analysis of variance of root dry matter at 67 DAP in the January 2008 experiment.

Square root transformation			
Source of Variation	df	MS	*p*-value
Identity	13	0.06	0.0067
Block	2	0.11	0.0080
Error	26	0.02	

Table 30. Mean of root dry matter at 48 DAP in the January 2008 experiment.

Square root transformation		Reversed transformation	
Identity	Root dry matter	Identity	Root dry matter (g plant^{-1})
MH 43-2	0.714	MH 43-2	0.510
R-99 OAC Rico	0.689	R-99 OAC Rico	0.475
PR 0340-3-3-1	0.682	PR 0340-3-3-1	0.465
VAX 3	0.642	VAX 3	0.412
Cardenal	0.603	Cardenal	0.364
Arroyo Loro Negro	0.600	Arroyo Loro Negro	0.360
PR 0443-151	0.591	PR 0443-151	0.349
RAB 655	0.586	RAB 655	0.343
OAC Rico	0.558	OAC Rico	0.311
PR 0427-7	0.543	PR 0427-7	0.295
A 774	0.519	A 774	0.269
Morales	0.508	Morales	0.258
Salagnac 90A	0.487	Salagnac 90A	0.237
PR 0422-41	0.474	PR 0422-41	0.225
Mean	0.585		
LSD (0.05)	0.109		
CV (%)	10.446		

Table 31. Mean of root dry matter at 67 DAP in the January 2008 experiment.

Square root transformation		Reversed transformation	
Line	Root dry matter	Identity	Root dry matter (g plant^{-1})
MH 43-2	1.09	MH 43-2	1.19
PR 0340-3-3-1	1.03	PR 0340-3-3-1	1.06
VAX 3	1.02	VAX 3	1.04
Arroyo Loro Negro	0.99	Arroyo Loro Negro	0.98
PR 0443-151	0.98	PR 0443-151	0.96
A 774	0.96	A 774	0.92
RAB 655	0.95	RAB 655	0.90
R-99 OAC Rico	0.84	R-99 OAC Rico	0.71
Cardenal	0.80	Cardenal	0.64
Salagnac 90A	0.77	Salagnac 90A	0.59
OAC Rico	0.74	OAC Rico	0.55
PR 0427-7	0.72	PR 0427-7	0.52
Morales	0.71	Morales	0.50
PR 0422-41	0.67	PR 0422-41	0.45
Mean	0.88		
LSD (0.05)	0.23		
CV (%)	15.91		

There was no correlation between mean nodulation scores and root dry matter at 48 and 67 DAP. However, the lines with higher root dry matter should be able to occupy more soil volume and have a greater capacity to acquire N. Physiological factors like root length, coverage area and exudates can affect nodulation in common bean lines (Brockwell et al., 1995).

A square root transformation of shoot dry matter was necessary to fulfill the normality and variance homogeneity assumptions. There was no significant difference in square root transformed shoot dry matter at 48 DAP (Table 32). Park and Buttery (2006) did not find an effect of *Rhizobium* strains on plant dry matter accumulation. They also found similar amounts of plant dry matter accumulated at 23 DAP (Park and Buttery, 2006). Lines with superior shoot dry matter accumulation at 48 DAP were PR 0340-3-3-1 (6.1 g plant^{-1}), A 774 (5.7 g plant^{-1}), PR 0443-151 (5.6 g plant^{-1}), RAB 655 (5.5 g plant^{-1}) and VAX 3 (5.2 g plant^{-1}) (Table 34). Shoot dry matter accumulation ranged from 3.6 to 6.1 g plant^{-1}. Cordero-García (1979) reported that shoot dry matter at three weeks after planting ranged 1.7 to 4.6 g plant^{-1} with inoculation and 112 kg N ha^{-1}. Cordero-García (1979) also reported that inoculation and 56 kg N ha^{-1} produced shoot dry matter that ranged from 1.6 to 2.4 g plant^{-1}. There was a significant difference ($p<0.05$) in shoot dry matter (square root transformed) at 67 DAP (Table 33). Park and Buttery (2006) also found significant differences among bean lines in plant dry matter at 36 DAP. At 67 DAP, lines with the greatest shoot dry matter were VAX 3, A 774, and PR 0443-151 (Table 35). A 774 and PR 0443-151 were superior lines in shoot dry matter at 48 and 67 DAP (Tables 34 and 35). Shoot dry matter of these superior lines ranged 8-11 g plant^{-1} (Table 35). Fernández-Toledo (1996) reported that beans at the Isabela Substation without inoculation and N fertilization produced shoot dry matter values ranging from 6 to 11 g plant^{-1}. PR 0340-3-3-1 and VAX 3 were superior in both root and shoot dry matter accumulation at both sampling dates (48 and 67 DAP).

Table 32. Analysis of variance of shoot dry matter at 48 DAP in the January 2008 experiment.

Square root transformation			
Source of Variation	df	MS	*p*-value
Identity	13	0.09	0.8659
Block	2	0.09	0.5793
Error	26	0.17	

Table 33. Analysis of variance of shoot dry matter at 67 DAP in the January 2008 experiment.

Square root transformation			
Source of Variation	df	MS	*p*-value
Identity	13	0.80	0.0146
Block	2	1.52	0.0130
Error	26	0.29	

Table 34. Mean of shoot dry matter at 48 DAP in the January 2008 experiment.

Square root transformation		Reversed transformation	
Identity	Shoot dry matter	Identity	Shoot dry matter (g plant^{-1})
PR 0340-3-3-1	2.47	PR 0340-3-3-1	6.10
A 774	2.39	A 774	5.71
PR 0443-151	2.36	PR 0443-151	5.57
RAB 655	2.35	RAB 655	5.52
VAX 3	2.28	VAX 3	5.20
Cardenal	2.26	Cardenal	5.11
PR 0427-7	2.24	PR 0427-7	5.02
MH 43-2	2.12	MH 43-2	4.49
OAC Rico	2.10	OAC Rico	4.41
Salagnac 90A	2.09	Salagnac 90A	4.37
Morales	2.07	Morales	4.28
Arroyo Loro Negro	2.02	Arroyo Loro Negro	4.08
PR 0422-41	1.95	PR 0422-41	3.80
R-99 OAC Rico	1.89	R-99 OAC Rico	3.57
Mean	2.18		
LSD (0.05)	NS		
CV (%)	18.81		

Table 35. Mean of shoot dry matter at 67 DAP in the January 2008 experiment.

Square root transformation		Reversed transformation	
Identity	Shoot dry matter	Identity	Shoot dry matter (g plant^{-1})
VAX 3	3.31	VAX 3	10.96
A 774	3.27	A 774	10.69
PR 0443-151	3.16	PR 0443-151	9.99
MH 43-2	3.10	MH 43-2	9.61
PR 0340-3-3-1	3.00	PR 0340-3-3-1	9.00
RAB 655	2.83	RAB 655	8.01
Cardenal	2.51	Cardenal	6.30
Arroyo Loro Negro	2.42	Arroyo Loro Negro	5.86
R-99 OAC Rico	2.30	R-99 OAC Rico	5.29
PR 0427-7	2.28	PR 0427-7	5.20
Morales	2.23	Morales	4.97
Salagnac 90A	2.14	Salagnac 90A	4.58
OAC Rico	1.87	OAC Rico	3.50
PR 0422-41	1.80	PR 0422-41	3.24
Mean	2.59		
LSD (0.05)	0.91		
CV (%)	20.99		

There were significant differences (*p*<0.05) among lines in root to shoot ratio at 48 DAP, but not at 67 DAP (Tables 36 and 37). At 48 DAP, the superior lines were R-99 OAC Rico, MH 43-2, Cardenal, RAB 655, and VAX 3 with ratios of 0.096, 0.084, 0.081, 0.081, and 0.80, respectively (Table 38). Dawson et al. (2008) suggested that plants with bigger root to shoot ratio were adapted better to low N soil conditions. At 67 DAP, the line with superior root to shoot ratios were Arroyo Loro Negro (0.18) and OAC Rico (0.17) (Table 39). In a period of 19 days these lines increased 2.6 and 2.4 times the root to shoot ratio, respectively.

Table 36. Analysis of variance of root to shoot ratio at 48 DAP in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Identity	13	0.0003	0.0444
block	2	0.0001	0.4686
Error	23	0.0001	

Table 37. Analysis of variance of root to shoot ratio at 67 DAP in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Identity	13	0.002	0.0641
Block	2	0.002	0.1569
Error	26	0.001	

Table 38. Mean of root to shoot ratio at 48 DAP in the January 2008 experiment.

Identity	Root:Shoot ratio
R-99 OAC Rico	0.0955
MH 43-2	0.0839
Cardenal	0.0812
RAB 655	0.0808
VAX 3	0.0802
PR 0340-3-3-1	0.0763
Arroyo Loro Negro	0.0743
OAC Rico	0.0713
A 774	0.0707
Salagnac 90A	0.0685
PR 0443-151	0.0645
Morales	0.0618
PR 0422-41	0.0591
PR 0427-7	0.0590
Mean	0.0734
LSD (0.05)	0.0212
CV (%)	16.428

Table 39. Mean of root to shoot ratio at 67 DAP in the January 2008 experiment.

Identity	Root:Shoot Ratio
Arroyo Loro Negro	0.180
OAC Rico	0.173
PR 0422-41	0.146
Salagnac 90A	0.143
R-99 OAC Rico	0.141
MH 43-2	0.125
PR 0340-3-3-1	0.119
RAB 655	0.114
Morales	0.106
Cardenal	0.105
PR 0427-7	0.102
PR 0443-151	0.101
VAX 3	0.099
A 774	0.089
Mean	0.124
LSD (0.05)	NS
CV (%)	27.759

There was no correlation between mean nodulation scores and root to shoot ratio at 48 and 67 DAP. There was no significant difference among lines in pod dry matter accumulation at 67 DAP (Table 40). There was no difference, but A 774 tend to have a higher pod dry matter weight (Table 41).

Table 40. Analysis of variance of pod dry matter at 67 DAP in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	20.21	0.1460
Block	2	38.56	0.0635
Error	26	12.55	

Table 41. Mean of pod dry matter at 67 DAP in the January 2008 experiment.

Line	Pod dry matter (g plant^{-1})
A 774	10.4
Arroyo Loro Negro	9.5
PR 0340-3-3-1	9.3
PR 0443-151	9.1
VAX 3	8.0
RAB 655	7.9
Salagnac 90A	7.7
MH 43-2	5.8
PR 0422-41	5.4
PR 0427-7	5.1
Cardenal	4.3
Morales	4.3
R-99 OAC Rico	3.3
OAC Rico	2.1
Mean	6.6
LSD (0.05)	NS
CV (%)	53.8

There was a significant difference (*p*<0.05) among lines in shoot (including pods) dry matter (Table 42). The lines with the greatest amounts of shoot dry matter were A 774 (21 g plant^{-1}), PR 0443-151 (19 g plant^{-1}), VAX 3 (19 g plant^{-1}), and PR 0340-3-3-1 (19 g plant^{-1}) (Table 43).

Table 42. Analysis of variance of shoot and pod dry matter at 67 DAP in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	72.13	0.0334
Block	2	126.20	0.0295
Error	26	31.19	

Table 43. Mean of shoot and pod dry matter at 67 DAP in the January 2008 experiment.

Line	Shoot and Pod dry matter (g plant^{-1})
A 774	21.7
PR 0443-151	19.4
VAX 3	19.0
PR 0340-3-3-1	18.9
RAB 655	16.1
Arroyo Loro Negro	15.6
MH 43-2	15.5
Salagnac 90A	12.5
Cardenal	10.7
PR 0427-7	10.6
Morales	9.3
PR 0422-41	9.0
R-99 OAC Rico	8.7
OAC Rico	5.8
Mean	13.8
LSD (0.05)	9.4
CV (%)	40.6

There was no correlation among bean lines between mean nodulation scores and shoot dry matter at 48 DAP. There were also no correlations among bean lines between mean nodulation score and shoot dry matter, pod dry matter and shoot and pod dry matter at 67 DAP. Some authors reported that a difference in nodule number had no effect on plant dry matter accumulation (Rennie and Kemp 1983; Kimani and Tongoona, 2008).

There was a significant difference ($p<0.05$) among bean lines in mean number of seeds per plant (Table 44). PR 0443-151 (33 seeds plant^{-1}), PR 0340-3-3-1 (27 seeds plant^{-1}), PR 0427-7 (26 seeds plant^{-1}), Arroyo Loro Negro (26 seeds plant^{-1}), and A 774 (25 seeds plant^{-1}) were superior lines (Table 45).

Table 44. Analysis of variance of number of seeds plant^{-1} at 67 DAP in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	86.58	0.0210
Block	2	69.13	0.1438
Error	22	32.60	

Table 45. Mean of number of seeds plant^{-1} at 67 DAP in the January 2008 experiment.

Line	Mean number of seed plant^{-1}
PR 0443-151	33.2
PR 0340-3-3-1	26.8
PR 0427-7	26.3
Arroyo Loro Negro	25.7
A 774	25.3
VAX 3	22.8
MH 43-2	22.2
RAB 655	21.7
Morales	19.8
Salagnac 90A	18.7
R-99 OAC Rico	16.3
OAC Rico	16.0
Cardenal	14.8
PR 0422-41	11.0
Mean	21.5
LSD (0.05)	10.2
CV (%)	26.4

There was no significant difference among lines in number of pods per plant at 67 DAP (Table 46), although the number of pods per plant ranged from 2.8 to 5.0 (Table 47). The mean number of pods plant^{-1} was 4.1, which is similar to 4.8 pods plant^{-1} reported by Araújo et al. (2000). PR 0443-151 and PR 0340-3-3-1 had the greatest number of seeds and pods plant^{-1} (Tables 45 and 47).

Table 46. Analysis of variance of number of pods plant^{-1} at 67 DAP in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Identity	13	1.23	0.2758
Block	2	1.33	0.2630
Error	22	0.94	

Table 47. Mean of number of pods plant^{-1} at 67 DAP in the January 2008 experiment.

Identity	Mean number of pods plant^{-1}
PR 0443-151	5.00
PR 0340-3-3-1	5.00
PR 0427-7	5.00
A 774	4.50
Salagnac 90A	4.33
RAB 655	4.33
Arroyo Loro Negro	4.33
VAX 3	4.17
MH 43-2	4.00
Morales	3.67
R-99 OAC Rico	3.50
PR 0422-41	3.25
OAC Rico	3.17
Cardenal	2.75
Mean	4.07
LSD (0.05)	NS
CV (%)	23.72

There was a significant difference ($p<0.05$) among bean lines in number of seeds pod^{-1} (Table 48). PR 0443-151 and Arroyo Loro Negro had the greatest number of seed pod^{-1} (Table 49). The number of seed pod^{-1} ranged from 3.4 to 6.6 with a mean of 5.2 (Table 49). Araújo et al. (2000) and Wahab et al. (1985) reported similar values of mean number of seeds per pod. PR 0443-151 was the only line superior in all seed yield components (number of seeds per plant, pods per plant and seeds per pod).

Table 48. Analysis of variance of number of seeds pod^{-1} at 67 DAP in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	1.53	0.0001
Block	2	0.42	0.1813
Error	22	0.23	

Table 49. Mean of number of seeds pods^{-1} at 67 DAP in the January 2008 experiment.

Line	Mean number of seed pod^{-1}
PR 0443-151	6.6
Arroyo Loro Negro	6.0
A 774	5.6
Morales	5.5
VAX 3	5.5
Cardenal	5.4
PR 0340-3-3-1	5.4
MH 43-2	5.3
PR 0427-7	5.2
RAB 655	5.0
OAC Rico	4.9
R-99 OAC Rico	4.6
Salagnac 90A	4.3
PR 0422-41	3.4
Mean	5.2
LSD (0.05)	0.9
CV (%)	9.1

There was a significant difference ($p<0.05$) among lines in seed yield (Table 50). PR 0340-3-3-1 (997 kg ha^{-1}), PR 0443-151 (876 kg ha^{-1}), VAX 3 (809 kg ha^{-1}), and RAB 655 (808 kg ha^{-1}) had superior yield (Table 51). These lines had seed yields significantly greater than A 774; [a line reported by Singh et al. (2003) to be tolerant to low soil fertility]. The mean seed yield in this field experiment ranged from approximately 150 to 1,000 kg ha^{-1} (Table 51). Miranda and Bliss (1991) reported variability on seed yield in two BNF experiments. Only four of the fourteen (29%) lines had a grain yield greater than 800 kg ha^{-1}. Araújo et al. (2000) obtained similar seed yields in a field trial in Brazil that received a low rate (30 kg ha^{-1}) of N fertilization. Several factors such as soil-borne pathogens from continuous cropping with beans, weed competition and drought stress may have contributed to the lower seed yields in the last field experiment. Another factor was the nitrogen extracted from soil in the previous three plantings. Even though N fertilizer had been applied, that applied amount was only the half of what the common bean requires. Similar results were reported by Fernández-Toledo (1996). Seed yield in the second planting was reduced considerably with a little N application.

Table 50. Analysis of variance of seed yield in the January 2008 experiment.

Source of Variation	df	MS	*p*-value	Coef
Line	13	325352	<0.0001	
Block	4	36542	0.2480	
Plant number	1	446641	0.0001	10.44
Error	51	26143		

Table 51. Adjusted Means (LSMeans) of seed yield in the January 2008 experiment.

Line	Seed yield (kg ha^{-1})
PR 0340-3-3-1	997
PR 0443-151	876
VAX 3	809
RAB 655	808
A 774	724
Arroyo Loro Negro	548
Cardenal	512
MH 43-2	508
PR 0427-7	490
PR 0422-41	438
R-99 OAC Rico	344
Morales	301
OAC Rico	249
Salagnac 90A	147
Mean	554
LSD (0.05)	205
CV (%)	29

The means of seed yield and seed yield components are presented in Table 52. There was a significant ($p<0.05$) and positive (r=0.76) correlation between seed yield and number of seeds per plant. There was also a significant ($p<0.05$) and positive correlation (r=0.73) between the seed yield and number of pods per plant. Wahab et al. (1985) found a positive correlation between yield and pods per plant. This yield component had a stronger association with seed yield than seeds per plant. There was a significant ($p<0.05$) and positive correlation (r=0.61) between the yield and seeds per pod in the January 2008 experiment. This yield component had a strong association with seed yield. Wahab et al. (1985) did not find a positive correlation between yield and seeds per pod.

There was no significant correlation between pods per plant and nodulation scores at 48 DAP and between seeds per plant and nodulation score at 48 DAP in the January 2008 experiment. There was no correlation between seed yield and nodulation score at 48 and 67 DAP at the January 2008 experiment. There was no correlation between nodulation score at 0, 25, and both N levels (kg ha^{-1}) in the June 2007 experiment with seed yield in the January 2008 experiment. There was no correlation between nodulation score at the February 2007 experiment and seed yield at the January 2008 experiment. In future investigations, common bean residues after harvest should be left on the field to improve nodulation. Dakora and Phillips (2002) reported that compounds exuded during vegetative tissue decomposition such as roots, nodules, and leaves can maintain the flavonoids concentration and mineral nutrition in the rhizosphere.

Table 52. Mean number of seeds plant^{-1}, pods plant^{-1}, seeds pod^{-1}, and seed yield in the January 2008 experiment.

Identity	Seeds plant^{-1}	Pods plant^{-1}	Seeds pod^{-1}	Seed yield (kg ha^{-1})
PR 0340-3-3-1	26.8	5.0	5.4	997
PR 0443-151	33.2	5.0	6.6	876
VAX 3	22.8	4.2	5.5	809
RAB 655	21.7	4.3	5.0	808
A 774	25.3	4.5	5.6	724
Arroyo Loro Negro	25.7	4.3	6.0	548
Cardenal	14.8	2.8	5.4	512
MH 43-2	22.2	4.0	5.3	508
PR 0427-7	26.3	5.0	5.3	490
PR 0422-41	11.0	3.3	3.4	438
R-99 OAC Rico	16.3	3.5	4.6	344
Morales	19.8	3.7	5.5	301
OAC Rico	16.0	3.2	4.9	249
Salagnac 90A	18.7	4.3	4.3	147
Mean	21.5	4.1	5.2	554
LSD (0.05)	10.2	NS	0.9	205
CV (%)	26.4	23.7	9.1	29.2

A square root transformation of a hundred seed weight was necessary to fulfill the normality and variance homogeneity assumptions. There was a significant difference ($p<0.05$) among bean lines for a hundred seed weight (square root transformed) (Table 53). Seven lines had a hundred seed weight > 25 g (medium seed size) (Table 54) (Van-Schoonhoven and Pastor-Corrales, 1987). The remaining lines had seed size < 25 g (Table 54).

Table 53. Analysis of variance of a hundred seed weight in the January 2008 experiment.

Square root transformation			
Source of Variation	df	MS	*p*-value
Line	13	0.90	<0.0001
Block	2	0.02	0.2141
Error	25	0.02	

Table 54. Mean of a hundred seed weight in the January 2008 experiment.

Square root transformation		Reversed transformation	
Line	Hundred seed weight	Line	Hundred seed weight (g)
PR 0422-41	6.26	PR 0422-41	39.19
Salagnac 90A	5.44	Salagnac 90A	29.59
VAX 3	5.21	VAX 3	27.14
RAB 655	5.17	RAB 655	26.73
Cardenal	5.15	Cardenal	26.52
PR 0340-3-3-1	5.09	PR 0340-3-3-1	25.91
A 774	5.02	A 774	25.20
Arroyo Loro Negro	4.91	Arroyo Loro Negro	24.11
MH 43-2	4.89	MH 43-2	23.91
PR 0427-7	4.54	PR 0427-7	20.61
PR 0443-151	4.49	PR 0443-151	20.16
Morales	4.46	Morales	19.89
R-99 OAC Rico	4.24	R-99 OAC Rico	17.98
OAC Rico	4.14	OAC Rico	17.14
Mean	4.93		
LSD (0.05)	0.21		
CV (%)	2.49		

There was no significant difference among bean lines in shoot N concentration (Table 55). The mean shoot N concentration was 2.4 (%) (Table 56) and similar to 2.6 (%) reported by Araújo et al. (2000). There was a significant (*p*<0.05) difference among bean lines in shoot N content (Table 57). VAX 3 (44.6 kg ha^{-1}), A 774 (43.5 kg ha^{-1}), PR 0340-3-3-1 (41.0 kg ha^{-1}), and MH 43-2 (40.1 kg ha^{-1}) had the greatest shoot N content (Table 58). These lines also produced higher biomass. There was no significant difference among bean lines in pod N concentration (Table 59). At 67 DAP, the common bean lines appear to have translocated N from shoot to pod with equal efficiency (Table 60). There was a significant ($p<0.05$) difference among bean lines in pod N content (Table 61). Arroyo Loro Negro (51.5 kg ha^{-1}), A 774 (44.0 kg ha^{-1}), and PR 0340-3-3-1 (40.5 kg ha^{-1}) produced the greatest pod N content (Table 62). In general, A 774 and PR 0340-3-3-1 were superior lines in shoot and pod N vegetative content at 67 DAP.

There was a significant ($p<0.05$) difference among bean lines in seed N concentration (%) (Table 63). The best lines were Salagnac 90A, PR 0422-41, and PR 0427-7 with seed N concentrations of 3.8, 3.6, and 3.5%, respectively (Table 64). PR 0427-7 combined good nodulation scores with among the greatest seed N concentrations. The percentage of seed N was similar to the values reported from trials conducted at the Isabela Substation by Catano-Catano (1990). Araújo et al. (2000) reported a mean seed N concentration (3.9% modified) similar to 3.4% (Table 64). There was a significant ($p<0.05$) difference in seed protein concentration (Table 65). This result is similar to seed N concentration because seed protein is calculated with a conversion factor of 6.25 (Riquelme, 1998). The superior lines in seed N concentration are the same as in seed protein (Table 66). The mean of seed protein was 21% (Table 66). This protein concentration is similar to those reported by Catano-Catano (1990) and Velázquez-Báez (1984).

There was a significant ($p<0.05$) difference among bean lines in seed N content (Table 67). PR 0443-151 (34 kg ha^{-1}), PR 0340-3-3-1 (30 kg ha^{-1}), and A 774 (26 kg ha^{-1}) produced the greatest seed N content (Table 68). Seed N content ranged from 2.0 to 34 kg ha^{-1} (Table 68). A large amount of diversity in acquiring atmospheric N and soil N has been reported for common bean (Rennie and Kemp, 1983; Kimani and Tongoona, 2008). Miranda and Bliss (1991) reported variability on total seed N in two BNF experiments. In Romania, Popescu (1998) reported a total N_2 fixed in seeds ranging from 30 to 70 kg ha^{-1}. PR 0443-151 had both the greatest seed yield (Table 51) and seed N content (Table 68). Miranda and Bliss (1991) found that selection for total seed N under low N soil conditions was effective for improving the BNF capacity of common bean. The lines with the best mean nodulation scores tended to have lower total N content (Table 68). A large number of nodules do not assure that a bean line will be efficient in the acquisition of N. In Mexico, Vásquez-Arroyo et al. (1998) reported similar results. Although a greater number of nodules do not always result in high N fixation, Vásquez-Arroyo et al. (1998) found a positive correlation between nodule number and N derived from the atmosphere in three bean cultivars evaluated in field trials in Mexico.

There was a significant ($p<0.05$) difference among bean lines in total N content (Table 69). Total N content ranged from 27 to 88 kg ha^{-1} (Table 70). Popescu (1998) reported N yield in total biomass ranging from 70 to 110 kg ha^{-1} with two *Rhizobium leguminosarum* bv. *phaseoli* strains in a Romanian soil. A 774 (88 kg ha^{-1}), PR 0340-3-3-1 (82 kg ha^{-1}), VAX 3 (75 kg ha^{-1}), and PR 0443-151 (69 kg ha^{-1}) produced the greatest total N content (Table 70). In a study by Kipe-Nolt and Giller (1993) bean lines with similar total N content fixed different amounts of N. There was a significant ($p<0.05$) difference among bean lines in the proportion of total N in the seed (Table 71). The proportion of total N yield in the seed ranged from 4.5 to 71.6% (Table 72). Morales (71.6%), PR 0443-151 (53.9%), Salagnac

90A (51.8%), and PR 0340-3-3-1 (47.0%) had the greatest proportion of seed N from the total N (Table 72). These lines were the most efficient in translocating the N from the shoot to the seed. Approximately 50% of the total N of PR 0443-151 and PR 0340-3-3-1 were translocated to the seed (Table 72). MH 43-2 and PR 0422-41 were the least efficient lines in translocating N from the shoot to the seed. Peña-Cabriales et al. (1993) found differences among bean lines in their ability to absorb and translocate N in the plant.

Table 55. Analysis of variance of shoot (stem and leaves) N concentration in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	0.13	0.4986
Block	2	0.34	0.0916
Error	26	0.13	

Table 56. Mean of shoot (stem and leaves) N concentration in the January 2008 experiment.

Line	Shoot N (%)
OAC Rico	2.88
PR 0422-41	2.56
MH 43-2	2.51
R-99 OAC Rico	2.49
Morales	2.43
VAX 3	2.42
Cardenal	2.39
PR 0340-3-3-1	2.37
PR 0427-7	2.33
RAB 655	2.31
A 774	2.31
Salagnac 90A	2.18
Arroyo Loro Negro	2.14
PR 0443-151	2.04
Mean	2.38
LSD (0.05)	NS
CV (%)	15.04

Table 57. Analysis of variance of shoot (stem and leaves) N content in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	339.60	0.0431
Block	2	892.99	0.0085
Error	26	155.09	

Table 58. Mean of shoot (stem and leaves) N content in the January 2008 experiment.

Line	Shoot N content (kg ha^{-1})
VAX 3	44.6
A 774	43.5
PR 0340-3-3-1	41.0
MH 43-2	40.1
PR 0443-151	36.4
RAB 655	31.6
Cardenal	25.2
PR 0427-7	22.8
Arroyo Loro Negro	22.5
R-99 OAC Rico	22.3
Morales	20.6
OAC Rico	18.2
Salagnac 90A	16.8
PR 0422-41	14.2
Mean	28.6
LSD (0.05)	20.9
CV (%)	43.6

Table 59. Analysis of variance of pod N concentration in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	0.12	0.0830
Block	2	0.06	0.3841
Error	23	0.06	

Table 60. Mean of pod N concentration in the January 2008 experiment.

Line	Pod N (%)
MH 43-2	3.0
Salagnac 90A	2.8
Cardenal	2.7
PR 0422-41	2.7
A 774	2.7
PR 0427-7	2.6
PR 0340-3-3-1	2.6
OAC Rico	2.6
R-99 OAC Rico	2.5
Arroyo Loro Negro	2.5
Morales	2.4
RAB 655	2.4
VAX 3	2.3
PR 0443-151	2.2
Mean	2.6
LSD (0.05)	NS
CV (%)	9.7

Table 61. Analysis of variance of pod N content in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	379.52	0.0408
Block	2	433.07	0.0960
Error	23	166.59	

Table 62. Mean of pod N content in the January 2008 experiment.

Line	Pod N content (kg ha^{-1})
Arroyo Loro Negro	51.5
A 774	44.0
PR 0340-3-3-1	40.5
PR 0422-41	34.8
Salagnac 90A	33.6
PR 0443-151	32.9
RAB 655	30.9
PR 0427-7	30.7
VAX 3	30.1
MH 43-2	28.9
Cardenal	19.3
Morales	17.6
R-99 OAC Rico	13.8
OAC Rico	8.4
Mean	29.8
LSD (0.05)	22.8
CV (%)	44.4

Table 63. Analysis of variance of seed N concentration in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	0.10	0.0083
Block	2	0.13	0.0341
Error	26	0.03	

Table 64. Mean of seed N concentration in the January 2008 experiment.

Line	Seed N (%)
Salagnac 90A	3.8
PR 0422-41	3.6
PR 0427-7	3.5
Morales	3.4
A 774	3.4
Arroyo Loro Negro	3.4
Cardenal	3.4
OAC Rico	3.4
R-99 OAC Rico	3.4
PR 0443-151	3.4
PR 0340-3-3-1	3.3
RAB 655	3.2
MH 43-2	3.2
VAX 3	3.1
Mean	3.4
LSD (0.05)	0.3
CV (%)	5.5

Table 65. Analysis of variance of seed protein concentration in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	4.09	0.0083
Block	2	5.18	0.0356
Error	26	1.36	

Table 66. Mean of seed protein concentration in the January 2008 experiment.

Line	Seed Protein (%)
Salagnac 90A	23.9
PR 0422-41	22.6
PR 0427-7	21.6
Morales	21.5
A 774	21.5
Arroyo Loro Negro	21.4
Cardenal	21.4
OAC Rico	21.1
R-99 OAC Rico	21.1
PR 0443-151	21.0
PR 0340-3-3-1	20.5
RAB 655	20.0
MH 43-2	19.8
VAX 3	19.1
Mean	21.2
LSD (0.05)	2.0
CV (%)	5.5

Table 67. Analysis of variance of seed N content in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	293.37	< 0.0001
Block	2	33.24	0.2450
Error	23	22.22	

Table 68. Mean of seed N content in the January 2008 experiment.

Line	Seed N content (kg ha^{-1})
PR 0443-151	33.8
PR 0340-3-3-1	29.6
A 774	26.1
Arroyo Loro Negro	25.1
Morales	24.7
RAB 655	22.9
Salagnac 90A	22.3
VAX 3	21.1
PR 0427-7	15.8
Cardenal	13.5
R-99 OAC Rico	7.2
OAC Rico	5.4
MH 43-2	3.9
PR 0422-41	2.0
Mean	18.1
LSD (0.05)	8.3
CV (%)	26.0

There was a significant (*p*<0.05) and positive (r=0.57) correlation between seed N content and total N content. There was no correlation between seed N content and pod N content, between shoot N content and pod N content, and between seed N content and shoot N content.

Table 69. Analysis of variance of total N content in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	1052.27	0.0494
Block	2	2249.66	0.0203
Error	26	495.10	

Table 70. Mean of total N content in the January 2008 experiment.

Identity	Total N content (kg ha^{-1})
A 774	87.51
PR 0340-3-3-1	81.51
VAX 3	74.68
PR 0443-151	69.30
MH 43-2	69.02
RAB 655	62.55
Arroyo Loro Negro	62.34
Salagnac 90A	50.40
PR 0427-7	45.90
Cardenal	44.53
PR 0422-41	38.81
Morales	38.13
R-99 OAC Rico	36.14
OAC Rico	26.60
Mean	56.24
LSD (0.05)	37.34
CV (%)	39.56

Table 71. Analysis of variance of the proportion of the total N in the seed in the January 2008 experiment.

Source of Variation	df	MS	*p*-value
Line	13	987.27	0.0005
Block	2	714.86	0.0517
Error	26	214.93	

Table 72. Mean of the proportion of the total N in the seed in the January 2008 experiment.

Identity	Proportion of the total N in seed (%)
Morales	71.61
PR 0443-151	53.87
Salagnac 90A	51.78
PR 0340-3-3-1	47.01
Arroyo Loro Negro	41.61
OAC Rico	37.02
PR 0427-7	34.13
Cardenal	32.66
VAX 3	31.07
RAB 655	29.56
A 774	28.62
R-99 OAC Rico	20.07
MH 43-2	5.57
PR 0422-41	4.54
Mean	34.94
LSD (0.05)	24.61
CV (%)	41.96

There was variation among bean lines in characteristics controlling N use efficiency (root and shoot dry matter, N content, and seed yield) which suggests that progress could be made in selection for these traits (Dawson et al., 2008). Due to the importance of genotype and environment interaction in the expression of BNF capacity, lines with good BNF need to be selected for particular agroecological conditions (Giller and Cadisch, 1995).

There has been a great amount of research that documents the benefits of inoculating common bean and other legumes with *Rhizobium*. The question arises why the inoculation of legumes with *Rhizobium* is not a common agricultural practice in Puerto Rico? Henzell (1988) stated that there was an increasing lack of knowledge of farmers concerning the fundamentals of the use of BNF in cropping systems. Bantilan and Johansen (1995) noted that the benefit promised by a new technology is realized when the technology is adopted and utilized by farmers. Henzell (1988) recommended an economic analysis of the inoculation to quantify the economic benefit of this technology. The inoculation of common bean with *Rhizobium* is an agricultural practice that should be implemented by the producers in Puerto Rico.

Conclusions

The white lines OAC Rico and PR 0427-7 had the best overall nodulation scores. The black bean line PR 0443-151 had the greatest mean seed yield in all experiments. PR 0427-7 and Arroyo Loro Negro combined good nodulation scores and seed yield potential. PR 0443-151 and PR 0340-3-3-1 had greater accumulation of N in the shoot and in the seed ana a greater translocation of the N from the shoot to the seed. These are traits needed to improve adaptation to the low N soil. PR 0443-151, Arroyo Loro Negro, and PR 0427-7 are recently released lines that were developed at the Isabela Substation. Because bean breeding lines at the Isabela Substation received a relatively low level of N fertilization (50 kg ha^{-1}) during their development, some indirect selection may have been made to increase N use efficiency or acquisition and possibly increase N fixation.

Once the benefits of inoculation have been established by research, a system needs to be established where bean producers have access to inoculums. For example, inoculums could be sold and distributed by the UPR Agricultural Experimental Station (AES) seed program. The AES Technical Guide for Bean Production should recommend inoculation and a split application of N with 25 kg N ha^{-1}. Additional evaluations need to be conducted in Puerto Rico to demonstrate the benefits of inoculation of bean seed with *Rhizobium*.

Recommendations

Even though common bean is a poor fixer of N, BNF can be improved through a breeding program (Hardarson, 1993). The BNF capacity of common bean can be improved using different techniques in a breeding program. More than one method for measuring N fixation should be used to estimate BNF capacity. These methods have been described in the scientific literature by Hardarson and Danso (1993), Herridge and Danso (1995), and Unkovich and Pate (2000). In the future, marker assisted selection may be used to enhance BNF (Nodary et al., 1993; Koinange et al., 1996). Santalla et al. (2001) suggested a strategy to enhance both the BNF capacity and grain yield in common bean breeding lines. Lines that where good nodulators (OAC Rico and PR 0427-7) and lines that were productive in low N soil (A 774, PR 0443-151, and PR 0340-3-3-1) could be released as cultivars or used as parents in a breeding program to increase yield potential, improve agronomical traits and enhance BNF (Hardarson et al., 1993).

Nutman (1984) concluded that characteristics such as early nodulation and more or larger nodules enhance BNF capacity of forage legumes in the temperate zone. Piha and Munns (1987) found that low BNF is caused by ineffective nodulation (small and numerous nodules). Nodulation has more impact on the fixation than the efficiency of the system itself. Nutman noted that a breeding program for increasing the BNF capacity should include: 1) increased seed yield under low N conditions (indirect selection), 2) persistent selection for soil nitrate tolerant lines capable of forming nodules and fix N with nitrate as a N source in the soil, and 3) the use of efficient *Rhizobium* strains and inoculation methods (Nutman, 1984). Herridge and Danso (1995) noted that in a breeding program it is very important to use the best and most efficient *Rhizobium* strain associated with a legume species. Henzell (1988) recommended selection of lines that nodulate better with native rhizobia or only with inoculants. The relative maturity of the line (Piha and Munns, 1987; Unkovich and Pate, 2000) and the below-ground N (including roots and nodules) (People and Herridge, 2000) should be considered when making selections for enhanced BNF.

R. leguminosarum biovar *phaseoli* was used as inoculant in this experiment, but other *Rhizobium* species such as *R. etli* and *R. tropici* strains should be used in future investigations. Grange et al. (2007) published that *R. etli* is the dominant species of *Rhizobium* in the two centers of origin (Mesoamerican and Andean) and that *R. tropici* is predominant in Brazil. Martínez-Romero et al. (1998) found that the number of nodules is dependant to the bean cultivar and the *Rhizobium* strain. They concluded that the cultivars BAT 477 and N-8-116 adapted better to strains of *Rhizobium etli* (Martínez-Romero et al., 1998). Bernal and Graham (2001) found that an efficient nodulation and fixation depended greatly on the bean cultivar and the *Rhizobium* strain. Ideally the bean line and the *Rhizobium* in BNF screening should have a similar origin. Vásquez-Arroyo et al. (1998) isolated native strains of *Rhizobium* in a Mexican soil and found that the greatest amount of the isolates were *Rhizobium etli*. In Brazil, Hungria et al. (2000) reported a *Rhizobium tropici* strain, PRF 81 (=SEMIA 4080), capable of producing common bean seed yields greater than 3,000 kg ha^{-1}.

Inoculation in this study was applied before covering the seed furrow. Different inoculation methods could be used to identify the most effective practice. Cordero-García (1979) made two types of inoculation (seed and furrow) to ensure the infection. Velázquez-Báez (1984) also used a soil and seed inoculation, but did not identify which was more effective. Meanwhile, in a soil with great population of native rhizobia, Popescu (1998) found that inoculation of the seed with a selected strain resulted favorable. Hardarson et al. (1989) found different biological N fixation and dry matter using different inoculations. A mixed culture of *R. tropici*, *R. leguminosarum*, and/or *R. etli* could be used as an inoculant (Araújo et al., 2000; Brockwell et al., 1995; Vadez et al. 1999) to identify which of the strains is more effective and compare it with native rhizobia. Manrique et al. (1993) used as inoculant a mixed of five local strains of *R. leguminosarum* bv. *phaseoli*. Inoculant in Puerto Rico should be specifically adapted for local conditions. This inoculant could be produced by the BNF laboratory at the University of Puerto Rico, Mayagüez Campus. Velázquez-Báez (1984) found a native strain of rhizobia (UPRM-6000) which was more competitive by producing highest percentage of nodules.

Rhizobium has a dual function providing N and protection from root rot to common bean. In combination with arbuscular mycorrhizae, the capacity of water absorption, nutrient acquisition (N and P) in low-fertile soils and protection from pathogens could be enhanced. Dakora and Phillips (2002) found that roots exudations benefit mycorrhizae formation. In future experiments the effect of rhizobia and mycorrhizae in common bean should be evaluated under low N and P soil conditions.

Bibliography

Aguilar, O.M., M.V. López and P.M. Riccillo. 2001. The diversity of rhizobia nodulating beans in Northwest Argentina as a source of more efficient inoculant strains. J. Biotech. 91:181-188.

Araújo, A.P., M.G. Texeira, and D.J. De Almeida. 2000. Growth and yield of common bean cultivars at two soil phosphorus levels under biological nitrogen fixation. Pesq. Agropec. Bras. 35(4):809-817.

Bantilan, M.C.S. and C. Johansen. 1995. Research evaluation and impact analysis of biological nitrogen fixation. Plant and Soil 174:279-286.

Beaver, J.S. 2006. Conjunto tecnológico de habichuela [online]. Available at http://academic.uprm.edu/jbeaver/index_files/CTBean/Outline%20.pdf (verified 20 Aug. 2006).

Beaver, J.S., J.C. Rosas, J. Myers, J. Acosta, J.D. Kelly, S. Nchimbi-Msolla, R. Misangu, J.Bokosi, S. Temple, E. Arnaud-Santana, and D.P. Coyne. 2003. Contributions of the Bean/Cowpea CRSP to cultivar and germplasm development in common bean. Field Crops Res. 82:87-102.

Bernal, G. and P.H. Graham. 2001. Diversity in the rhizobia associated with *Phaseolus vulgaris* L. in Ecuador and comparisons with Mexican bean rhizobia. Can. J. Microbiol. 47(6):526-534.

Bliss, F.A. 1993a. Breeding common bean for improved biological nitrogen fixation. Plant and Soil 152:71-79.

Bliss, F.A. 1993b. Utilizing the potential for increased nitrogen fixation in common bean. Plant and Soil 152:157-160.

Bliss, F.A. and J.C. Miller, Jr (ed.) 1988. Selecting and breeding grain legumes for enhanced nitrogen fixation. p. 1001-1012. *In* R.J. Summerfield (ed.) World crops: Cool season food legumes. Kluwer Academic Publishers, Netherlands.

Bøckman, O.C. 1997. Fertilizers and biological nitrogen fixation as sources of plant nutrients: Perspectives for future agriculture. Plant and Soil 194:11-14.

Brady, N.C. and R.R. Weil. 2002. The nature and properties of soils. 13th ed. Pearson Education, India.

Brockwell, J., P.J. Bottomley, and J.E. Thies. 1995. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: A critical assessment. Plant and Soil 174:143-180.

Casanoves, F., R. Macchiavelli, and M. Balzarini. 2005. Error variation in multienvironment peanut trials: Within-trial spatial correlation and between-trial heterogeneity. Crop Sci. 45:1927-1933.

Catano-Catano, H. 1990. Selección de genotipos de *Phaseolus vulgaris* capaces de nodular en presencia de fertilizante nitrogenado. M.S. Thesis. Univ. of Puerto Rico, Mayagüez, Puerto Rico. 55p.

Cordero-García, M. 1979. Efecto de la inoculación con *Rhizobium phaseoli* y la fertilización nitrogenada en el comportamiento de la habichuela seca, *Phaseolus vulgaris*. M.S. Thesis. Univ. of Puerto Rico, Mayagüez, Puerto Rico. 58p.

Dakora, F.D. and D.A. Phillips. 2002. Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant and Soil 245:35-47.

Da Silva, P.M., S.M. Tsai, and R. Bonetti. 1993. Response to inoculation and N fertilization for increased yield and biological nitrogen fixation of common Bean (*Phaseolus vulgaris* L.). Plant and Soil 152:123-130.

Date, R.A. 2000. Inoculated legumes in cropping systems of the tropics. Field Crops Res. 65:123-136.

Dawson, J.C., D.R. Huggins, S.S. Jones. 2008. Characterizing nitrogen use efficiency in natural and agricultural ecosystems to improve the performance of cereal crops in low-input and organic agricultural systems. Field Crops Res. 107:89-101.

Dean, J.R. and K.W. Clark. 1980. Effect of low level nitrogen fertilization on nodulation, acetylene reduction and dry matter in fababeans and three other legumes. Can. J. Plant. Sci. 60:121-130.

Departamento de Agricultura de Puerto Rico. 2007. Compendio Estadístico del Ingreso Bruto Agrícola desde 1980-81 al 2005-06. Oficina de Estadísticas Agrícolas, Estado Libre Asociado de Puerto Rico, San Juan, Puerto Rico.

De Oliviera, W.S., L.W. Meinhardt, A. Sessitsch, and S.M. Tsai. 1998. Analysis of *Phaseolus-Rhizobium* interactions in subsistence farming system. Plant and Soil 204:107-115.

Dorcinvil, R. 2009. Respuesta agronómica de líneas de habichuela (*Phaseolus vulgaris* L.) a la fertilidad de un Oxisol. M.S. Thesis. Univ. of Puerto Rico, Mayagüez, Puerto Rico. 149p.

Ehteshamul-Haque, S. 1994. Use of rhizobia in the control of soilborne plant diseases caused by root infecting fungi. Ph.D. Thesis. University of Karachi, Karachi-75270, Pakistan. 239p.

Epstein, E. and A.J. Bloom. 2005. Mineral nutrition of plants: Principles and perspectives. 2nd ed. Sinauer Associates, Sunderland, MA.

Fernández-Toledo, F.E. 1996. Evaluación de treinta genotipos de habichuela común (*Phaseolus vulgaris* L.) con tolerancia a calor para potencial de fijación de nitrógeno. M.S. Thesis. Univ. of Puerto Rico, Mayagüez Campus, Puerto Rico. 84p.

Food and Agricultural Organization (FAO). 2000. Overview and case studies on biological nitrogen fixation: Perspectives and limitations [online]. Available at http://www.fao.org/ag/agl/agll/soilbiod/cases/caseB1.pdf (verified 25 Feb. 2009).

Gepts, P and D. Debouck. 1991. Origin, domestication, and evolution of the common bean (*Phaseolus vulgaris* L.). p. 7-53. *In* A. Van-Schoonhoven and O. Voysest (ed.) Common beans research for crop improvement. CAB International, Wallingford, UK.

Giller, K.E. and G. Cadisch. 1995. Future benefits from biological nitrogen fixation: An ecological approach to agriculture. Plant and Soil 174:255-277.

Graham, P.H., J.C. Rosas, C. Estévez de Jensen, E. Peralta, B. Tlusty, J. Acosta-Gallegos, P.A. Arraes Pereira. 2003. Addressing edaphic constraints to bean production: the Bean/Cowpea CRSP project in perspective. Field Crops Res. 82:179-192.

Graham, P.H. and C.P. Vance. 2000. Nitrogen fixation in perspective: an overview of research and extension needs. Field Crops Res. 65:93-106.

Grange, L., M. Hungria, P.H. Graham, E. Matínez-Romero. 2007. New insights into the origins and evolution of rhizobia that nodulate common bean (*Phaseolus vulgaris*) in Brazil. Soil, Biol. & Biochem. 39:867-876.

Guadalupe-Luna, R. 1977. Influencia de la época de siembra en el comportamiento de la habichuela seca (*Phaseolus vulgaris*). M.S. Thesis. Univ. of Puerto Rico, Mayagüez Campus, Puerto Rico. 91p.

Hansen, A.P., P. Martin, B.R. Buttery, and S.J. Park. 1992. Nitrate inhibition of N_2 fixation in *Phaseolus vulgaris* L. cv. OAC Rico and a supernodulating mutant. New Phytol. 122:611-612

Hardarson, G. 1993. Methods for enhancing symbiotic nitrogen fixation. Plant and Soil 152:1-17.

Hardarson, G., F.A. Bliss, M.R. Cigales-Rivero, R.A. Enson, J.A. Kipe-Nolt, L. Longeri, A. Manrique, J.J. Peña-Cabriales, P.A.A. Pereira, C.A. Sanabria, and S.M. Tsai. 1993. Genotypic variation in biological nitrogen fixation by common bean. Plant and Soil 152:59-70.

Hardarson, G. and S.K.A. Danso. 1993. Methods for measuring biological nitrogen fixation in grain legumes. Plant and Soil 152:19-23.

Hardarson, G., M. Golbs, and S.K.A. Danso. 1989. Nitrogen fixation in soybean (*Glycine max* L. Merrill) as affected by nodulation patterns. Soil Biol. and Biochem. 21(6):783-787.

Havlin, J.L., J.D. Beaton, S.L. Tisdale, and W.L. Nelson. 1999. Soil fertility and fertilizers: An introduction to nutrient management. 6th ed. Prentice Hall, Upper Saddle River, NJ.

Henson, R.A. 1993. Measurements of N_2 fixation by common bean in Central Brazil as affected by different reference crops. Plant and Soil 152:53-58.

Henzell, E.F. 1988. The role of biological nitrogen fixation research in solving problems in tropical agriculture. Plant and Soil 108:15-21.

Herridge, D. and S.K.A. Danso. 1995. Enhancing crop legume N2 fixation through selection and breeding. Plant and Soil 174:51-82.

Herridge, D. and I. Rose. 2000. Breeding for enhanced nitrogen fixation in crop legumes. Field Crops Res. 65:229-248.

Huang, W. 2007. Impact of rising natural gas prices on U.S. ammonia supply. A report from the Economic Research Service, USDA. WRS-0702. August 2007. p 19.

Hungria, M. and M.A.T. Vargas. 2000. Environmental factors affecting N_2 fixation in grain legumes in the tropics, with an emphasis on Brazil. Field Crops Res. 65:151-164.

Hungria, M., M.A.T. Vargas, R.J. Campo, L.M.O. Chueire, and D.S. Andrade. 2000. The Brazilian experience with the soybean (*Glycine max*) and common bean (*Phaseolus vulgaris*) symbioses. p. 515-518. *In* F.O. Pedrosa et al. (eds.) Nitrogen fixation: From molecules to crop productivity. Kluwer Academic Publishers, Netherlands.

InfoStat. 2008. InfoStat versión 2008. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Ishizuka, J. 1992. Trends in biological nitrogen fixation research and application. Plant and Soil 141:197-209.

Jensen, E.S. and H. Hauggaard-Nielsen. 2003. How can increased use of biological N_2 fixation in agriculture benefit the environment? Plant and Soil 252:177-186.

Junta de Planificación. 2006. Plan de uso de terrenos de Puerto Rico, perfil regional, región oeste. http://jpops02.jp.gobierno.pr/pls/portal/docs/PAGE/PAGINASINICIO/PUTPR/DOCANEJ/REGI%D3N%20OESTE%20FINAL.PDF

Khalequzzaman, K.M. and I. Hossain. 2007. Effect of seed treatment with *Rhizobium* strains and biofertilizers on foot/root rot and yield of bushbean in *Fusarium solani* infested soil. J. Agric. Res. 45(2):151-160.

Kimani J.M. and P. Tongoona. 2008. The mechanism of genetic control for low soil nitrogen (N) tolerance in common bean (*Phaseolus vulgaris* L.). Euphytica 162:193-203.

Kipe-Nolt, J.A. and K.E. Giller. 1993. A field evaluation using the ^{15}N isotope dilution method of lines of *Phaseolus vulgaris* L. bred for increased nitrogen fixation. Plant and Soil 152:107-114.

Kipe-Nolt, J.A., H. Vargas, and K.E. Giller. 1993. Nitrogen fixation in breeding lines of *Phaseolus vulgaris* L. Plant and Soil 152:103-106.

Koinange, E.M.K., S.P. Singh, and P. Gepts. 1996. Genetic control of the domestication syndrome in common-bean. Crop Sci. 36:1037-1045.

Liu, L. C., R. Montalvo-Zapata, J. Ortiz-López, J.A. Rodríguez, and J. Aponte. 1999. Effect of planting dates and frequencies of intercropping on yield and income of bean and banana. J. Agric. Univ. Puerto Rico 83:209-216.

Liu, L. C., J.A. Rodríguez, and J. Ortiz. 1998. Intercropping bean cultivars with plantain. J. Agric. Univ. Puerto Rico 81:151-158.

Lynch, J.P. 2007. Roots of the second green revolution. Australian Journal of Botany 55:493-512.

Mangual-Crespo, G., R. Kluson, and E.C. Schröder. 1987. Nitrogen levels and Rhizobium inoculation and yields of native white bean (*Phaseolus vulgaris* L.). J. Agric. Univ. Puerto Rico 71(1):1-6.

Manrique, A., K. Manrique, and J. Nakahodo. 1993. Yield and biological nitrogen fixation of common bean (*Phaseolus vulgaris* L.) in Peru. Plant and Soil 152:87-91.

Martínez-Romero, E. 2003. Diversity of *Rhizobium-Phaseolus vulgaris* symbiosis: overview and perspectives. Plant and Soil 252:11-23.

Martínez-Romero, E., I. Hernández-Lucas, J.J. Peña-Cabriales, and J.Z. Castellanos. 1998. Symbiotic performance of some modified *Rhizobium etli* strains in assays with *Phaseolus vulgaris* beans that have a high capacity to fix N_2. Plant and Soil 204:89-94.

MDS Harris. 2007. Methods used by Harris Laboratory: Soil analysis, plant analysis, media testing [online]. Available at http://harristurf.crinet.com/lab_accuracy/procedures_methods.asp (verified 7 May 2009).

Miklas, P.N., J.D. Kelly, S.E. Beebe, and M.W. Blair. 2006. Common bean breeding for resistance against biotic and abiotic stresses: From classical to MAS breeding. Euphytica 147:105-131.

Miranda, B.D. and F.A. Bliss. 1991. Selection for increased seed nitrogen accumulation in common bean: Implications for improving dinitrogen fixation and seed yield. Plant Breeding 106:301-311.

Moawad, H., S.M.S. Badr El-Din, and R.A. Andel-Aziz. 1998. Improvement of biological nitrogen fixation in Egyptian winter legumes through better management of *Rhizobium*. Plant and Soil 204:95-106.

Müller, S., P.A.A. Pereira, and P. Martin. 1993. Effect of different levels of mineral nitrogen on nodulation and N_2 fixation of two cultivars of common bean (*Phaseolus vulgaris* L.). Plant and Soil 152:139-143.

Muthomi, J.W., P.E. Otieno, G.N. Chemining'wa, J.H. Nderitu, and J.M. Wagacha. 2007. Effect of legume root rot pathogens and fungicide seed treatment on nodulation and biomass accumulation. J. Biol. Sci. 7(7):1163-1170.

Nodari, R.O., S.M. Tsai, P. Guzmán, R.L. Gilbertson, and P. Gepts. 1993. Toward an integrated linkage map of common bean. III. Mapping genetic factors controlling host-bacteria interactions. Genetics 134:341-350.

Nutman, P.S. 1984. Improving nitrogen fixation in legumes by plant breeding; the relevance of host selection experiments in red clover (*Trifolium pratense* L.) and subterranean clover (*T. subterraneum* L.) Plant and Soil 82:285-301.

Pachico, D. 1999. Common bean: The nearly perfect food. In: CIAT in focus, may 1999. Cali, Colombia: Centro Internacional de Agricultura Tropical [online]. Available at http://www.ciat.cgiar.org/es/sala_not/pdf/Beanfocus.pdf (verified 23 Jan. 2009).

Park, S.J. and B.R. Buttery. 2006. Registration of ineffective nodulation mutant R69 and nonnodulation mutant R99 common bean genetic stocks. Crop Sci. 46:1415-1416.

Pedalino, M., K.E. Giller, and J. Kipe-Nolt. 1992. Genetic and physiological characterization of the non-nodulating mutant of *Phaseolus vulgaris* L.-NOD125. J. Exp. Bot. 43:843-849.

Peña-Cabriales, J.J. and J.Z. Castellanos. 1993. Effect of water stress on N_2 fixation and grain yield of *Phaseolus vulgaris* L. Plant and Soil 152:151-155.

Peña-Cabriales, J.J., O.A. Grageda-Cabrera, V. Kola, and G. Hardarson. 1993. Time course of N_2 fixation in common bean (*Phaseolus vulgaris* L.). Plant and Soil 152:115-121.

Peoples, M.B. and D.F. Herridge. 2000. Quantification of biological nitrogen fixation in agricultural systems. p. 519-524. *In* F.O. Pedrosa et al. (eds.) Nitrogen fixation: From molecules to crop productivity. Kluwer Academic Publishers, Netherlands.

Peoples, M.B., D.F. Herridge, and J.K. Ladha. 1995. Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production? Plant and Soil 174:3-28.

Piha, M.I. and D.N. Munns. 1987. Nitrogen fixation potential of beans (*Phaseolus vulgaris* L.) compared with other grain legumes under controlled conditions. Plant and Soil 98:169-182.

Popescu, A. 1998. Contributions and limitations to symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris* L.) in Romania. Plant and Soil 204:117–125.

Ramírez, G. 1984. Efecto de la fertilización con nitrógeno y fósforo del frijol común (*Phaseolus vulgaris*) en un suelo de Upala. Agron. Costarr. 8(1):69-73.

Raun, W.R. and G.V. Johnson. 1999. Improving nitrogen use efficiency for cereal production. Agron J. 91:357-363.

Rennie, R.J. and G.A. Kemp. 1983. N_2-fixation in field beans, quantified by ^{15}N isotope dilution. II. Effect of cultivars of beans. Agron. J. 75:645-649.

Riquelme, E. 1998. Manual del laboratorio de Nutrición. Laboratorio 3: Determinación de proteína bruta (Método Micro-Kjeldahl). p. 3-1, 3-2, and 3-3.

Saito, S.M., M. Nazareth, S. Montanheiro, R.L. Victoria, and K. Reichardt. 1984. The effects of N fertilizer and soil moisture on the nodulation and growth of *Phaseolus vulgaris* L. Agric. Sci. 103:87-93.

Santalla, M., J.M. Amurrio, A.P. Rodiño, and A.M. de Ron. 2001. Variation in traits affecting nodulation of common bean under intercropping with maize and sole cropping. Euphytica 122:243-255.

Sharif, T., S. Khalil, and S. Ahmad. 2003. Effect of *Rhizobium* sp., on growth of pathogenic fungi under *in vitro* conditions. Pak. J. Biol. Sci. 6(18): 1597-1599.

Singh, S.P., H. Terán, C.G. Muñoz, J.M. Osorno, J.C. Takegami, and M.D.T. Thung. 2003. Low soil fertility tolerance in landraces and improved common bean genotypes. Crop Sci. 43:110-119.

Singleton, P.W. and J.W. Tavares. 1986. Inoculation response of legumes in relation to the number and effectiveness of indigenous *Rhizobium* populations. Appl. and Environ. Microbiology 51(5):1013-1018.

Somasegaran, P. and H.J. Hoben. 1994. Handbook for rhizobia: Methods in legume-Rhizobium technology. Springer-Verlag, NY, Inc.

Sotomayor-Ramírez, D. 2006. Calibración de pruebas de suelo [online]. Available at http://academic.uprm.edu/dsotomayor/agro6505/AGRO6505_Notas_4A.pdf (verified 7 May 2009).

Sotomayor-Ramírez, D. and G.A. Martínez. 2006. The status of phosphorus and other fertility paramenters in soils of Puerto Rico. J. Agric. Univ. Puerto Rico 90(3-4):145-157.

Taiz, L. and E. Zeiger. 2002. Plant physiology. 3rd ed. Sinauer Associates, Sunderland, MA.

Tang, C., P. Hinsinger, B. Jaillard, Z. Rengel, and J.J. Drevon. 2001. Effect of phosphorus deficiency on the growth, symbiotic N2 fixation and proton release by two bean (*Phaseolus vulgaris*) genotypes. Agronomie 21:683-689.

Triplett, E.W., B.G. Rolfe, E.C. Cocking, I. Kennedy, and J. Vanderleyden. 2007. A nitrogen story: Extending the productive associations of nitrogen-fixing bacteria can reduce cost of food production in an era of high oil prices and concern over food security. Microbe 2(8):372-373.

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

Unkovich, M.J. and J.S. Pate. 2000. An appraisal of recent field measurements of symbiotic N_2 fixation by annual legumes. Field Crops Res. 65:211-228.

Vadez, V., J.H. Lasso, D.P. Beck, and J.J. Drevon. 1999. Variability of N_2-fixation in common bean (*Phaseolus vulgaris* L.) under P deficiency is related to P use efficiency. Euphytica 106:231-242.

Van-Schoonhoven, A. and M.A. Pastor-Corrales. 1987. Sistema estándar para la evaluación de germoplasma de frijol. CIAT (Centro Internacional de Agricultura Tropical) (ed.), Cali, Colombia. 56p.

Vargas, M.A.T., I.C. Mendes, and M. Hungria. 2000. Response of field-grown bean (*Phaseolus vulgaris* L.) to *Rhizobium* inoculation and nitrogen fertilization in two Cerrados soils. Biol. Fertil. Soils 32:228-233.

Vásquez-Arroyo, J., A. Sessitsch, E. Martínez, and J.J. Peña-Cabriales. 1998. Nitrogen fixation and nodule occupancy by native strains of *Rhizobium* on different cultivars of common bean (*Phaseolus vulgaris* L.). Plant and Soil 204:147-154.

Velázquez-Báez, Y.A. 1984. Respuesta de la habichuela (*Phaseolus vulgaris* L.) variedad Arroyo Loro No. 1 a la inoculación con *Rhizobium* phaseoli en Lajas, PuertoRico. M.S. Thesis. Univ. of Puerto Rico, Mayagüez Campus, Puerto Rico. 43p.

Velázquez, Y.A, R.A. Kluson, and E.C. Schröder. 1988. Rhizobium inoculation of *Phaseolus vulgaris* in Lajas, Puerto Rico. J. Agric. Univ. Puerto Rico 72:427-436.

Wahab, A.H., E. Montague-Gordon, J. Dehaney, A.L. Wright, and M.A. Lugo-López. 1985. Performance of eleven dry bean cultivars (*Phaseolus vulgaris*) over two successive seasons on the hillsides of Jamaica. J. Agric. Univ. P.R. 69(3):245-254.

Wani, S.P., O.P. Rupela, and K.K. Lee. 1995. Sustainable agriculture in the semi-arid tropics through biological nitrogen fixation in grain legumes. Plant and Soil 174: 29-49.

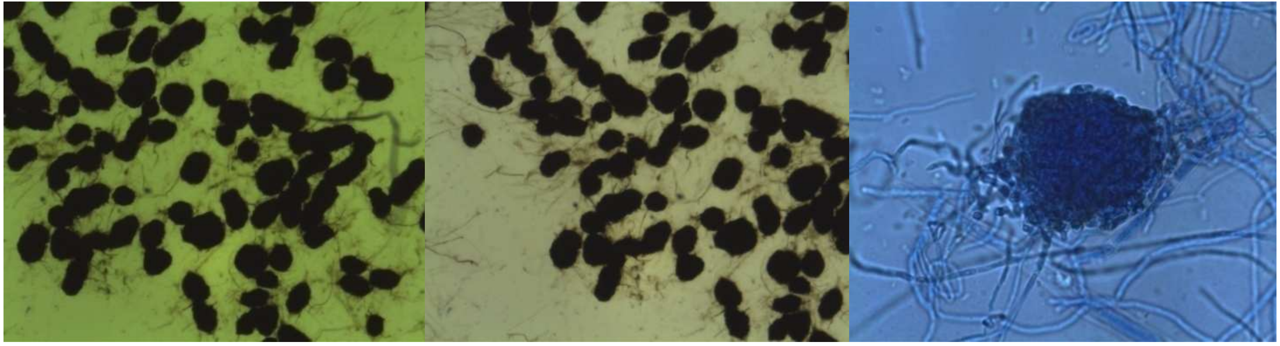
Winarno, R. and T.A. Lie. 1979. Competition between *Rhizobium* strains in nodule formation: Interaction between nodulating and non-nodulating strains. Plant and Soil 51:135-142.

Zahran, H.H. 1999. *Rhizobium*-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol. Mol. Biol. Rev. 63(4):968-989.

Zurdo-Piñeiro, J.L., E. Velázquez, M.J. Lorite, G. Brelles-Mariño, E.C. Schröder, E.J. Bedmar, P.F. Mateos, and E. Martínez-Molina. 2004. Identification of fast-growing Rhizobia nodulating tropical legumes from Puerto Rico as *Rhizobium gallicum* and *Rhizobium tropici*. System. Appl. Microbiol. 27:469-477.

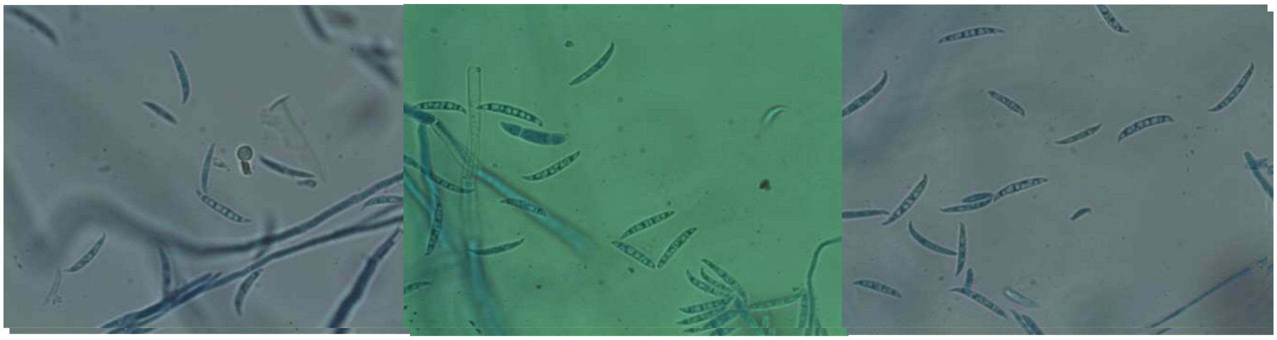
Appendix

Appendix 1. Images of *Macrophomina phaseolina*.



Acknowledgement Lydia Rivera, Ph.D. professor of the Department of Crop Protection.

Appendix 2. Images of *Fusarium* sp.



Acknowledgement Luz M. Serrato, M.S. student of the Department of Crop Protection.