



## Performance of Mesoamerican bean (*Phaseolus vulgaris* L.) lines in an unfertilized oxisol<sup>1</sup>

### Comportamiento de líneas mesoamericanas de frijol (*Phaseolus vulgaris* L.) en un oxisol no fertilizado

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## Abstract

**Introduction.** Common beans (*Phaseolus vulgaris* L.) in Central America and the Caribbean are often produced on low fertility soils which reduces crop yield. Bean breeding programs need to identify genotypes that have superior adaptation to these conditions. **Objective.** Identify Mesoamerican bean germplasm lines with superior adaptation to low soil fertility. **Materials and methods.** The performance of twenty-seven Mesoamerican bean (*Phaseolus vulgaris* L.) lines from the Bean Abiotic Stress Evaluation (BASE) 120 panel were evaluated in an unfertilized oxisol at Isabela, Puerto Rico over five growing seasons (four-year period from 2015-2018). The lines were inoculated with a mixture of *Rhizobium etli* and *R. tropici* to promote symbiotic nitrogen fixation (SNF). **Results.** Four lines produced mean seed yields >1,200 kg ha<sup>-1</sup> and had estimates of nitrogen derived from the atmosphere (Ndfa) >50 %. Greater nodule number was positively correlated with % Ndfa, later maturity and seed yield. The heat and drought tolerant small red cultivar ‘Rojo Chortí’ and the heat tolerant white cultivar ‘Verano’ had among the smallest apparent C isotope discrimination values suggesting greater water use efficiency. Among the elite lines in the trial, root rot damage was minimal and the basal root growth angles were intermediate (40-60 %), which favored the uptake of water and soil nutrients. **Conclusion.** Mesoamerican bean lines with superior seed yield and enhanced symbiotic nitrogen fixation in a low fertility soil were identified. Many of these lines also possess resistance to other biotic and abiotic factors that limit bean seed yield in Central America and the Caribbean.

**Keywords:** plant breeding, soil fertility, symbiotic nitrogen fixation, *Rhizobium*, carbon isotope discrimination.



## Resumen

**Introducción.** El frijol común (*Phaseolus vulgaris* L.) en América Central y el Caribe a menudo se produce en suelos de baja fertilidad que reduce el rendimiento del cultivo. Los programas de mejoramiento de frijoles necesitan identificar genotipos que tengan una adaptación superior a estas condiciones. **Objetivo.** Identificar líneas de germoplasma de frijol mesoamericano con una adaptación superior a la baja fertilidad del suelo. **Materiales y métodos.** El comportamiento de veintisiete líneas de frijol mesoamericano (*Phaseolus vulgaris* L.) del Vivero de Adaptación de Frijol a Estrés Abióticos (BASE 120), fue evaluado en un oxisol sin fertilizante químico en Isabela, Puerto Rico, durante cinco épocas de siembra (periodo de cuatro años de 2015-2018). Las líneas se inocularon con una mezcla de *Rhizobium etli* y *R. tropici* para promover la fijación simbiótica de nitrógeno (SNF). **Resultados.** Cuatro líneas produjeron rendimientos promedios de semilla >1200 kg ha<sup>-1</sup> y presentaron estimaciones de nitrógeno derivado de la atmósfera (Ndfa) >50 %. El mayor número de nódulos se correlacionó positivamente con % Ndfa, días a la madurez y rendimiento de semilla. El cultivar rojo pequeño tolerante al calor y la sequía ‘Rojo Chortí’ y el cultivar blanco ‘Verano’ tolerante al calor, presentaron los valores de discriminación de isótopos de C más bajos que sugieren una mayor eficiencia en el uso del agua. El daño por pudrición de la raíz fue mínimo y los ángulos de crecimiento basal de la raíz fueron intermedios (40-60 %), lo que favoreció la absorción de agua y nutrientes del suelo en las líneas élites del ensayo. **Conclusión.** Se identificaron líneas de frijol mesoamericanas con un rendimiento superior y una mejor fijación simbiótica de nitrógeno en un suelo de baja fertilidad. Algunas líneas también poseen resistencias a otros factores bióticos y abióticos que limitan el rendimiento de semilla de frijol en Centroamérica y el Caribe.

**Palabras clave:** fitomejoramiento, fertilidad del suelo, fijación simbiótica de nitrógeno, *Rhizobium*, discriminación de isótopos de carbono.

## Introduction

The production of Mesoamerican (black, small red, and white) beans (*Phaseolus vulgaris* L.) in Central America and the Caribbean (CAC) is threatened by several diseases and pests including *Bean golden yellow mosaic virus*, *Bean common mosaic virus*, *Bean common mosaic necrosis virus*, rust caused by *Uromyces appendiculatus* (Pers.: Pers.) Unger, common bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* and leafhoppers (*Empoasca* spp.) (Beaver et al., 2003; Rosas et al., 2000; Rosas, 2011). Considerable progress has been made within the region in the development and release of Mesoamerican bean cultivars having enhanced levels of disease resistance to these and other biotic constraints (Beaver et al., 2018a, 2018b; Porch et al. 2014; Rosas et al., 2004). Bean producers in Central America and the Caribbean also face numerous abiotic yield constraints including terminal drought, high temperatures, and infertile soils (Miklas et al., 2006). Beans in Latin America and the Caribbean are often produced by farmers with limited resources on degraded soils that are deficient in N (Beebe et al., 2012). Low levels of soil P can reduce nodule numbers and increase the proportion of nodules that are ineffective for symbiotic N<sub>2</sub> fixation (SNF) (Pereira & Bliss, 1987).

The combination of enhanced SNF with the capacity to better acquire or utilize soil N should improve adaptation of beans to unfertile soils. Bean genotypes that combine greater SNF and nitrogen use efficiency would reduce both dependence on N fertilizer and potential contamination of N in the environment (Akter et al., 2018).

Among the grain legumes, common bean has the lowest levels of SNF with an average percentage of nitrogen derived from the atmosphere (Ndfa) of 40 % (Herridge et al., 2008). Nitrogen fixation in common bean can be limited by energy supply to the nodules (Graham et al., 2003). Assuming that additional factors such as low soil P are not limiting, bean lines in low N soils that have greater %Ndfa may have the capacity to partition greater

amounts of photosynthate to roots. This may lead to a temporary deficiency in the availability of photosynthates for growth and development of the aerial portion of the plant (Oldroyd & Leyser, 2020). Chlorotic leaves are a common symptom of soil N deficiency. A positive correlation has been reported between leaf N content and SPAD scores in bean leaves at the V4 stage (vegetative) of development (Abrahão et al., 2013). A positive association between SPAD at flowering and %NDFa was reported in field trials conducted in Ontario, Canada (Farid et al., 2017).

Although efforts to improve symbiotic nitrogen capacity of beans have been successful, the time and cost of phenotypic screening limits routine selection for this complex trait (Kamfwa et al., 2015). Indirect selection for enhanced symbiotic nitrogen fixation might be possible when breeding lines are evaluated in low N soils. Because bean breeding programs in Central America and the Caribbean often screen lines on research stations and farms having poor soil fertility, some selection of bean lines having improved SNF or greater acquisition of nutrients from the soil may have occurred (Graham et al., 2003). Under drought conditions, bean genotypes that combine high seed yield and larger  $\Delta^{13}\text{C}$  values often have deeper roots and lower water use efficiency and may represent the “water user” response to drought stress (Polania et al., 2016a; Sanz-Saez et al., 2019).

This study evaluated the performance of a group of elite Mesoamerican bean breeding lines and cultivars that were selected, in large part, for disease resistance in an unfertilized oxisol at Isabela, Puerto Rico having low levels of soil N. The objective of this research was to identify elite Mesoamerican bean germplasm with superior adaptation to low soil fertility. This germplasm can be used as parents for the continued genetic improvement of Mesoamerican beans for Central America and the Caribbean.

## Materials and methods

The performance of twenty-seven elite lines of common bean (*Phaseolus vulgaris* L.) from the Bean Abiotic Stress Evaluation (BASE) 120 panel (Oladzad et al., 2019) was evaluated at the Isabela Substation of the Agricultural Experiment Station of the University of Puerto Rico over a four-year period (2015 to 2018). The Substation is located on the northwestern coastal plain of Puerto Rico at 18.468 N, -67.042 W at an altitude of 128 m. The average annual minimum and maximum temperatures at the Isabela Experimental Substation are 22.2 and 27.8 °C, with an average annual rainfall of 1,630 mm.

The soil where the field trials were performed is a Coto Clay, a very fine, kaolinitic, isohyperthermic Typic Eutruxox. Soil samples were taken from sites where the BASE 120 trials were conducted. Soil sampling consisted of taking samples at different points in the experimental sites using a zig-zag sampling pattern. A soil sampling tube was used to take sub-samples at a depth of 30 cm in the soil. The top 5 cm of soil was removed to eliminate plant material that could contaminate the sample. The subsamples were mixed to prepare a composite sample for each site. The soil chemical analyses were performed at AgSource Harris Laboratories in Lincoln, NE, to determine the availability of soil nutrients (Table 1). Soil pH was measured in a 1:1 soil:water ratio; soil organic matter was measured by loss on ignition; available phosphorus was extracted with Bray 1 if pH was  $\leq 7.2$ , followed by quantification with Inductively Coupled Plasma Spectroscopy (ICP) and exchangeable cations (potassium), using ammonium acetate extraction followed by quantification with ICP.

The trials were conducted at the Isabela Substation during five growing seasons. The trials were planted in June and November 2015, June 2016, June 2017, and June 2018. In order to evaluate the ability of bean genotypes to nodulate and establish symbiotic nitrogen fixation in a soil having low N fertility, no fertilization was applied to the BASE 120 trials.

Except for the June 2017 planting date, field trials included 118 common bean genotypes and two tepary bean (*Phaseolus acutifolius* L.) lines from the BASE 120 panel (Tables 2 and 3). The June 2017 planting included 27 elite lines from the BASE 120 panel. Entries in the BASE 120 panel included elite bean breeding lines and cultivars

**Table 1.** Results from soil tests from the sites where the tests were carried out with bean (*Phaseolus vulgaris* L.) lines of the Bean Abiotic Stress Evaluation panel (BASE 120) trials. Isabela, Puerto Rico, from 2015 to 2018.

**Cuadro 1.** Resultados de los análisis de suelo de los sitios donde se realizaron los ensayos con líneas de frijol (*Phaseolus vulgaris* L.) del Panel de Evaluación de Estreses Abióticos del Frijol (BASE 120). Isabela, Puerto Rico, entre 2015 y 2018.

Trial planting date	pH	Organic matter -%-	Nitrate	Phosphorus -ppm-	Potassium
Jun. 2015	6.6	4.4	23.0	16.6	239
Nov. 2015	6.6	4.2	8.0	11.3	177
Jun. 2016	5.7	4.1	4.1	7.4	93
Jun. 2017	7.2	4.4	1.0	18.3	176
Jun. 2018	6.6	3.9	11.0	4.2	59

**Table 2.** Seed type, pedigree, and traits of elite bean (*Phaseolus vulgaris* L.) lines from the Bean Abiotic Stress Evaluation panel (BASE 120) evaluated in Isabela, Puerto Rico, over five planting dates between 2015 to 2018.

**Cuadro 2.** Tipo de semilla, pedigrí y características de líneas élite de frijol (*Phaseolus vulgaris* L.) del Panel de Evaluación de Estreses Abióticos del Frijol (BASE 120) evaluadas en Isabela, Puerto Rico, durante cinco fechas de siembra, entre 2015 y 2018.

Line	Seed type	Pedigree	Traits	Reference
TARS-MST1	Black	'Negro Tacana'/VAX 6	<i>I</i> , SAP6, SU91, heat and drought tolerance, root rot resistance	Porch et al. (2012)
PR1418-15	Black	PR0443-151/'Verano'	Low soil fertility tolerance	Granadino-Espinal & León-Gonzalez (2016)
PR1483-105	Black	'Verano'//DPC-40/'Zorro'	<i>bgm-1</i> , SW12, <i>I</i>	Granadino-Espinal & León-Gonzalez (2016)
PR1147-1 (Hermosa)	Black	'Negro Veracruz' / PR9607-29// 'VAX 6 / MUS 83 // DOR482 / BAT 93'	<i>bgm-1</i> , SW12, <i>I</i> , <i>bc-3</i>	Beaver et al. (2018)
B12724	Black	B09184/B09135	<i>I</i> , SU91	
BIOF 4-70	Black	G23818B/EAP9503-32B	<i>bgm-1</i> , SW12, <i>I</i> , low soil fertility tolerance	
'Sayaxché'	Black	DOR 390/'Tío Canela 75'// SRC1-1-18/'Milenio'	<i>bgm-1</i> , SW12, <i>I</i> , low soil fertility and drought tolerance	Instituto de Ciencia y Tecnología Agrícolas (2010)
MHN 322-49	Black	'ICTA Ligero'/MH2-2	<i>bgm-1</i> , SW12, <i>I</i> , ashy stem blight and common bacterial blight resistance, drought tolerance	
'Sankara' ('Azabache 40')	Black	DOR483/BelNeb RR-2// MUS83/DOR483/'Raven'	<i>bgm-1</i> , SW12, <i>I</i> , <i>bc-3</i> , angular leaf spot resistance	Beaver et al. (2014) Rosas et al. (2016)
PR1165-3	Black	DPC-40/'Zorro'	<i>bgm-1</i> , <i>I</i> , <i>bc-3</i> , long and dense root hairs	
'Beníquez'	White	DOR483/BelNeb RR-2// MUS83/DOR483/'Raven'	<i>bgm-1</i> , SW12, <i>I</i> , <i>bc-3</i> , heat tolerance	Beaver et al. (2011)
'ICA Pijao'	Black	CIAT germplasm G5773 from Colombia	<i>I</i> , symbiotic nitrogen fixation	
'Lenca Precoz'	Black	'ICTA Ligero'/'Raven'	<i>bgm-1</i> , SW12, <i>I</i> , <i>bc-3</i> , early maturity, drought tolerance	Rosas et al. (2016)

**Table 3.** Seed type, pedigree, and traits of elite bean (*Phaseolus vulgaris* L.) lines from the Bean Abiotic Stress Evaluation panel (BASE 120) evaluated in Isabela, Puerto Rico, over five planting dates between 2015 to 2018.**Cuadro 3.** Tipo de semilla, pedigrí y características de líneas élite de frijol (*Phaseolus vulgaris* L.) del Panel de Evaluación de Estreses Abióticos del Frijol (BASE 120) evaluadas en Isabela, Puerto Rico, durante cinco fechas de siembra entre 2015 y 2018.

Line	Seed type	Pedigree	Traits (References)
'Bella'	White	'Verano'//PR0003-124/'Raven'	<i>bgm-1</i> , SW12, <i>I</i> , <i>bc-3</i> , common blight resistance (Beaver et al., 2018a)
SB2-170	Cream	'Matterhorn'/G21212///'Matterhorn'/DOR 364//USPT-ANT1/H405-8-1-1	Drought tolerance
Paisano PF (MER 2212-28)	Small red	'Milenio'/'Amadeus 77'	<i>bgm-1</i> , SW12, <i>I</i> , heat tolerance
FBN 1203-43	Small red	'Amadeus 77'/'Amadeus77'/'Paraisito'///'Amadeus 77'/IBC 306-55	<i>bgm-1</i> , SW12, <i>I</i> , SNF, long and dense root hairs
'INTA Centro Sur' (IBC 301-204)	Small red	'Amadeus 77'/'Amadeus 77'/'Paraisito'	<i>bgm-1</i> , SW12, <i>I</i> , SNF, low soil fertility tolerance
TARS-LFR1	Small red	BAT 477/VAX 2//BAT 477/VAX 1/3/VAX 3 using recurrent selection	<i>I</i> , root rot resistant, SNF, low soil fertility tolerance (Porch et al., 2014)
'CENTA Pipil' ('Rojo INIFAP')	Small red	'Bribri'/MD 30-37//RS3	<i>bgm-1</i> , SW12, <i>I</i> , heat tolerance (Centro Nacional de Tecnología Agropecuaria y Forestal, 2005; Villar-Sánchez et al., 2010)
SER 118	Small red	SXB 123/EAP9503-32B//RCB137	Drought tolerance, superior performance in low P soils
FBN 1203-47	Small red	'Amadeus 77'/'Amadeus77'/'Paraisito'///'Amadeus 77'/IBC 306-55	<i>bgm-1</i> , SW12, <i>I</i> , SNF
'Rojo Chorti'('CENTA EAC')	Small red	'Negro Vaina Blanca'/BCN 20-0294	<i>bgm-1</i> , SW12, <i>I</i> , heat tolerance (Rosas et al., 2019; Parada-Cardona et al., 2015)
BAT 477	Cream	51052/ICA Bunsii/51052/Cornell 49-242	<i>I</i> , tolerance to low soil fertility, root rot resistance, plastic gravitropic root systems – shallower root systems in low P soils (Liao et al., 2001)
'Verano'	White	DOR 364/WBB-20-1/'Don Silvio'/VAX 6	<i>bgm-1</i> , SW12, <i>I</i> , common blight resistance (Beaver et al., 2008)
'Beníquez'	White	DOR483/BelNeb RR-2//MUS83/DOR483///'Raven'	<i>bgm-1</i> , SW12, <i>I</i> , <i>bc-3</i> , heat tolerance (Beaver et al., 2011)
'Paraisito Mejorado 2-Don Rey'	Small red	'Carrizalito' *2/'Paraisito'	<i>bgm-1</i> , SW12, <i>I</i> , tolerance to drought and low soil fertility tolerance (Rosas, 2015)
SEF 16	Small red	ALB 74/INB 841//RCB 593	<i>bgm-1</i> , <i>I</i> , terminal drought and heat tolerance (Chaves-Barrantes, 2015)

from Zamorano University in Honduras, the International Center for Tropical Agriculture (CIAT), the University of Puerto Rico, USDA-ARS Tropical Agriculture Research Station and Michigan State University.

Although no fertilizer was applied in the elite BASE 120 trials, the lines were inoculated with two strains of *Rhizobium*; *R. etli* (CIAT 632) and *R. tropici* (CIAT 899), that was prepared at the Juana Diaz Station Laboratory. During the June 2015 planting date, a liquid suspension of inoculum at a concentration of  $10^7$  rhizobia cells per mm was applied first directly to the seeds in the row and, after emergence, to the base of the seedlings using a backpack sprayer. The estimated amount of liquid inoculant was based on the volume recommended for 50 kg of seeds and 14 seeds per m. The inoculation for the other growing seasons was performed using a solid inoculant.

This peat-based inoculant contained the same two strains of *Rhizobium*. The procedure consisted of applying 25 g of *Rhizobium* inoculant and PREMAX (bacterial protector) per 1 kg of seeds. The inoculant was applied to each genotype by stirring the inoculant and the seed of each genotype in a plastic container. The inoculant had a concentration of  $1 \times 10^9$  rhizobia  $\text{g}^{-1}$  peat. When using this inoculation technique, it was necessary to ensure that the seeds were evenly covered by the inoculant. The inoculated seed were stored at room temperature, overnight and planted the following day.

The entries from the BASE 120 trials (Tables 2 and 3) were planted in a randomized complete block design with five replications. The experimental units were single 3 m rows with 0.76 m spacing between experimental units. The seeding rate was 14 seeds per m. The field trials received supplementary aerial irrigation to avoid drought stress. Weeds were controlled manually. The trials were monitored twice a week to detect the presence and incidence of pests and diseases. Preventive measures were taken in cases where a high density of pests was found. No fungicides were applied to the field trials.

Nodulation was evaluated during flowering (approximately 45 days after planting) using two plants per experimental unit. Plant root crowns were carefully extracted at about 0.75 m from the end of each plot. Root crowns were washed to remove the excess soil. Nodule numbers were evaluated by assigning values from 1-9 using the CIAT scale, where 1 represented a plant with >81 nodules, 3 had 41-80 nodules, 5 had 21-40 nodules, 7 had 10-20 nodules, and 9 had <10 nodules per plant (van Schoonhoven & Pastor-Corrales, 1987). The chlorophyll content was measured approximately 45-50 days after planting using a Minolta Chlorophyll Meter SPAD-502 on three randomly chosen central leaves of plants from each experimental unit.

Seed samples from each of three replications of the BASE 120 trials conducted at the Isabela Substation in 2016, 2017, and 2018 were used to estimate percentage of N derived from the atmosphere (%Ndfa) and apparent C isotope discrimination ( $\Delta^{13}\text{C}$ ). Approximately 5 seed of each sample were dried until a constant weight was achieved at 70 °C, and then were ground at the USDA-ARS Tropical Agriculture Research Station using a Wiley mini-mill (Thomas Scientific, Swedesboro, New Jersey, USA), passed through a #40 mesh sieve, resulting in a fine powder. The 4.2 mg samples were packaged in 5x8 mm tin capsules (D1008, EA Consumables, Pennsauken, NJ) and shipped to the University of California, Davis Stable Isotope Facility in 96-well plates.

The  $^{15}\text{N}$  natural abundance method (Unkovich et al., 2008) was used to calculate the %Ndfa estimates. The white bean R-99 was used as the non-N-fixing reference line.

$$\%Ndfa = [(\Delta^{15}\text{N R99} - \Delta^{15}\text{N BASE 120 line}) / (\Delta^{15}\text{N R99-B})] * 100$$

Where B represents the  $\Delta^{15}\text{N}$  of the bean line grown under N-free conditions and relies on symbiotic nitrogen fixation for all N requirements. In this study, the variable B was assigned the value of 0 because the R99 reference line does not nodulate. The entries in the BASE 120 trial and the R99 reference bean line have similar patterns of phenological development and seed size. In a N-free trial conducted by Heilig et al. (2017), no significant differences in  $\Delta^{15}\text{N}$  were found among flowering plants of ‘Puebla 152’, ‘Zorro’, ‘Medalist’, and PR0443-151. Constant B values for seed of 2.44 0/00 for bean genotypes having type II and 2.88 0/00 for genotypes having type III growth habits were used for greenhouse studies conducted by CIAT in Cali, Colombia (Polania et al., 2016b). The use of a constant B value, however, would not change the ranks of %Ndfa estimates among the entries. The size of the B value is less important when %Ndfa estimates are low (<50 %) (Unkovich, 2008). The  $^{15}\text{N}$  natural abundance method using seed tissue is well-suited for use by a breeding program to screen for superior SNF (Polania et al., 2016b).

Carbon isotope discrimination ( $\Delta^{13}\text{C}$ , ‰) estimates were calculated using the following equation:

$$\Delta^{13}\text{C} = (\Delta^{13}\text{C}_{\text{atmosphere}} - \Delta^{13}\text{C}_{\text{sample}}) / (\Delta^{13}\text{C}_{\text{sample}} + 1)$$

Where  $\Delta^{13}\text{C}_{\text{atmosphere}}$  ( $-8\text{‰}$ ) is the C isotope composition of  $\text{CO}_2$  in the atmosphere (Farquhar et al., 1989) and  $\Delta^{13}\text{C}_{\text{sample}}$  is the C isotope composition of the seed sample.

Data collected from the 27 lines were used for the analyses. Statistical analysis was performed using a GLIMMIX model from SAS/STAT 14.3 (SAS Institute, Cary, NC) to analyze main effects and interactions and to compare the least squares means. Planting dates and bean genotypes were considered as fixed effects whereas replication was considered a random effect within planting dates. A 95 % probability level was used to establish statistical significance. Pearson correlations were calculated to study associations between least squares means of seed yield and other traits measured in this study.

## Results

Soil pH in the fields at the Isabela Substation where the BASE 120 trials were conducted ranged from 5.7 to 6.6 and soil organic matter ranged from 3.9 to 4.4, which are adequate values for normal bean growth and development (Table 1). Three of the planting dates had soil nitrate levels  $<10$  ppm and a fourth date had 11 ppm of soil nitrate. Results from soil samples taken for the June 2018 planting had a sub-optimum level of P. The soil samples taken for the other planting dates had medium to high levels of P and K for dry bean production.

The pedigrees in Tables 2 and 3 show that a diverse group of Mesoamerican parents was used to develop the elite breeding lines in this study. The pedigrees, however, include very few parents from other bean races and no Andean bean parents.

Fourteen of the 27 lines produced mean seed yields  $>1,000$  kg ha<sup>-1</sup> in un-fertilized trials planted at the Isabela Substation over five growing seasons (Table 4). In order to adapt to existing cropping systems in CAC, the elite lines have Type II and III growth habits and reached harvest maturity from 73.4 to 87.0 days after planting (Table 5).

Significant genotype x season (GxE) interactions for seed yield were observed for most bean lines. One of the exceptions was the line SB2-170 that combined a mean seed yield  $>1,000$  kg ha<sup>-1</sup> and non-significant GxE across environments. The black bean cultivar ‘Hermosa’ produced among the highest mean seed yields although nodulation scores were poor and %NDFa estimates were low.

Good root health is needed to efficiently absorb available soil nutrients and water. Mean root rot scores were low ( $<3$ ) suggesting that the lines had good root health in these trials, thus this was not a factor limiting nutrient uptake (Table 5). Mean basal root growth angle (BRGA) ranged from 54.1 to 64.6 % (Table 5). GxE for BRGA, except for TARS-MST1 and PR1483-105, was non-significant for the higher yielding lines. The eight highest-yielding lines also had non-significant GxE for SPAD, %NDFa, and root rot scores (Table 6).

SPAD scores ranged from 37.1 to 50.1 (Table 6). Five lines had nodulation scores  $<6.0$ , although the five lines with the highest mean seed yields had nodulation scores  $>6.0$  (Table 6). In contrast, four of the five lines with the highest seed yields had %NDFa  $>50$  % (Table 6).

Seed yield and harvest date were not correlated. Nodulation scores were negatively correlated with harvest date ( $-0.48^*$ ) but positively associated with seed yield ( $0.39^*$ ). Thus, the later maturing lines and cultivars in the study tended to have greater nodule numbers although a few of the highest yielding lines had fewer nodules. Nodule number, however, does not reflect the effectiveness of nodules to fix nitrogen. In this study, nodule number and %NDFa were positively associated reflected by a negative correlation ( $-0.40^*$ ) between nodulation scores ( $1 \geq 81$  and  $9 < 10$  nodules/plant) and %NDFa estimates. %NDFa was not significantly associated with days to maturity or seed yield. There was a negative correlation ( $-0.53^{**}$ ) between SPAD scores and %NDFa.

**Table 4.** Least square means of seed yield of elite bean (*Phaseolus vulgaris* L.) lines from the Bean Abiotic Stress Evaluation panel (BASE 120) evaluated in Isabela, Puerto Rico over five growing seasons from 2015 to 2018.**Cuadro 4.** Medias ajustadas de rendimiento de semillas de líneas elite de frijol (*Phaseolus vulgaris* L.) del Panel de Evaluación de Estreses Abióticos del Frijol (BASE 120), evaluadas durante cinco fechas de siembra en Isabela, Puerto Rico entre 2015 y 2018.

Line	BASE 120 entry number	June 2015	Nov. 2015	June 2016	June 2017	June 2018	Mean	F Test for season comparisons within each line
Bella	70	1652	869	1574	1329	955	1276	**
TARS-MST1	111	1083	902	1729	1494	1024	1246	**
PR1418-15	71	1606	598	1732	1386	889	1242	**
PR1483-105	72	1418	679	1725	1329	978	1226	**
‘Hermosa’	64	1540	1020	1249	1349	889	1209	**
B12724	6	1699	412	1362	1249	1295	1203	**
BIOF 4-70	20	983	718	1561	1428	1289	1196	**
SB2-170	109	1391	834	1304	1093	1097	1144	NS
‘Sayaxché’	78	1326	641	1502	1299	856	1125	**
MER 2212-28	54	942	723	1516	1115	1302	1120	**
FBN 1203-43	36	1291	365	1476	1042	1205	1076	**
‘INTA Centro Sur’	44	1039	603	1393	1020	1286	1068	**
TARS-LFR1	110	1011	799	1379	1185	897	1054	NS
‘CENTA Pipil’	30	1246	672	1404	1056	852	1046	**
SER 118	98	449	463	1681	1022	1005	924	**
MHN 322-49	55	807	731	1095	879	1096	922	NS
‘Sankara’	118	349	919	1401	971	844	897	**
FBN 1203-47	37	919	250	1190	913	1199	894	**
‘Rojo Chortí’	102	672	347	1429	886	1003	867	**
BAT 477	10	853	428	1310	930	815	867	**
‘Verano’	117	420	724	1492	979	710	865	**
PR1165-3	68	353	497	1472	824	884	806	**
‘Beníquez’	12	433	415	1493	781	584	741	**
‘Paraisito Mej. 2’	59	562	330	765	762	789	642	NS
SEF 16	92	347		1202	762	625	635	**
‘ICA Pijao’	43	396	531	887	630	673	623	NS
‘Lenca Precoz’	53	429	460	834	565	628	583	NS

F Tests for comparing season means sliced by lines for bean lines having superior %Ndfa were not significant, suggesting that this trait was stable across environments.

The small red bean, ‘Rojo Chortí’, consistently had the lowest  $\Delta^{13}\text{C}$  CID values over the three growing seasons (Table 7). The black bean lines B12724, PR1165-3, and ‘Sankara’ had among the largest least squared means for  $\Delta^{13}\text{C}$  across growing seasons. The black bean lines TARS-MST1 and B12724 were ranked in the top 6 in seed yield

**Table 5.** Least square means of seed yield, SPAD scores, nodulation scores, root rot scores, basal root growth angle (BRGA), and harvest date of elite bean (*Phaseolus vulgaris* L.) lines from the Bean Abiotic Stress Evaluation panel (BASE 120) evaluated in Isabela, Puerto Rico. 2015-2018.

**Tabla 5.** Medias ajustadas de rendimiento de semilla, lecturas de pudrición de la raíz, BRGA y fecha de cosecha de líneas élite de frijol (*Phaseolus vulgaris* L.) del Panel de Evaluación de Estrés Abiótico del Frijol (BASE 120), evaluadas en Isabela, Puerto Rico. 2015-2018.

Line	Seed yield (kg ha <sup>-1</sup> )	Root rot score (1-9)	BRGA (%)		Harvest date		
	Mean	Mean	F Test for season comparisons in each entry	Mean	F Test for season comparisons in each entry	Mean	F Test for season comparisons in each entry
‘Bella’	1276	2.1	NS	62.8	NS	81.0	**
TARS-MST1	1246	1.6	NS	61.0	*		
PR1418-15	1242	2.2	NS	57.3	NS	80.1	**
PR1483-105	1226	2.0	NS	58.5	*	78.6	**
‘Hermosa’	1209	1.6	NS	60.8	NS	75.5	NS
B12724	1203	2.0	NS	59.5	NS	81.4	**
BIOF 4-70	1196	2.0	NS	56.0	NS	76.9	**
SB2-170	1144	1.8	NS	59.8	NS	75.2	NS
‘Sayaxché’	1125	1.9	NS	62.0	NS	81.1	**
MER 2212-28	1120	2.0	*	62.3	*	78.3	**
FBN 1203-43	1076	2.0	**	59.2	NS	75.0	**
‘INTA Centro Sur’	1068	2.2	*	64.6	NS	77.2	**
TARS-LFR1	1054	1.7	NS	58.8	NS		**
‘CENTA Pipil’	1046	2.3	*	59.0	*	78.0	
SER 118	924	1.9	*	55.5	NS	74.8	**
MHN 322-49	922	1.5	NS	63.5	NS	87.0	**
‘Sankara’	897	2.0	NS	55.9	NS		
FBN 1203-47	894	2.4	NS	59.3	NS	76.9	**
BAT 477	867	1.8	NS	62.2	NS	80.4	**
‘Rojo Chortí’	867	2.2	**	61.8	**	83.7	NS
‘Verano’	865	1.8	NS	54.8	*		
PR1165-3	806	1.6	*	54.1	**	81.3	**
‘Benítez’	741	2.1	NS	54.2	*	83.6	**
‘Paraisito Mej. 2’	642	1.9	**	61.3	*	84.2	**
SEF 16	635	1.9	*	57.4	NS	74.7	**
‘ICA Pijao’	623	2.1	NS	62.7	NS	84.5	**
‘Lenca Precóz’	583	2.1	NS	63.7	NS	73.4	**

over 5 growing seasons and showed high  $\Delta^{13}\text{C}$  values. The white bean cultivar ‘Verano’ and the small red breeding line TARS-LFR1 had among the lowest  $\Delta^{13}\text{C}$  values across seasons. Both lines were selected in Puerto Rico for adaptation to higher temperatures when daily water demand is the highest, thus lower  $\Delta^{13}\text{C}$  values in this study may be associated with water use efficiency.

**Table 6.** Least square means of seed yield, SPAD scores, nodulation scores, and % nitrogen derived from the atmosphere (%Ndfa) of elite bean (*Phaseolus vulgaris* L.) lines from the Bean Abiotic Stress Evaluation panel (BASE 120) evaluated in Isabela, Puerto Rico, from 2015 to 2018.

**Tabla 6.** Medias ajustadas de rendimiento de semilla, lecturas de SPAD, lecturas de nodulación y (% nitrógeno derivado de la atmósfera (% Ndfa) de líneas élite de frijol (*Phaseolus vulgaris* L.) del Panel de Evaluación de Estrés Abióticos del Frijol (BASE 120), evaluadas en Isabela, Puerto Rico, entre 2015 y 2018.

Line	Seed yield (kg ha <sup>-1</sup> )	SPAD score	Nodulation score (1-9)		Ndfa (%)		
	Mean	Mean	F Test for season comparisons in each entry	Mean	F Test for season comparisons in each entry	Mean	F Test for season comparisons in each entry
'Bella'	1276	46.4	NS	6.3	**	55.0	NS
TARS-MST1	1246	40.5	NS	7.0	NS	53.2	NS
PR1418-15	1242	40.9	NS	6.2	*	58.8	NS
PR1483-105	1226	43.7	NS	6.7	NS	52.3	NS
'Hermosa'	1209	48.4	NS	7.6	NS	23.1	NS
B12724	1203		NS	6.8	**	44.6	NS
BIOF 4-70	1196	38.1	NS	7.4	NS		
SB2-170	1144	50.1	NS	7.5	NS	42.7	NS
'Sayaxché'	1125	46.4	*	6.6	**		
MER 2212-28	1120	49.6	NS	7.2	NS	27.9	NS
FBN 1203-43	1076	45.4	NS	6.9	NS	33.8	NS
'INTA Centro Sur'	1068	40.3	NS	5.9	**	56.0	NS
TARS-LFR1	1054	40.4	NS	6.9	*	43.8	NS
'CENTA Pipil'	1046	47.1	NS	7.4	*	42.1	NS
SER 118	924	45.9	NS	7.5	*	39.6	**
MHN 322-49	922	44.1	NS	6.4	*	29.7	NS
'Sankara'	897	42.6	NS	5.7	*	37.4	NS
FBN 1203-47	894	39.8	NS	5.9	NS	55.1	NS
BAT 477	867	37.1	NS	6.8	**	58.0	NS
'Rojo Chortí'	867	45.0	NS	6.6	*	53.7	NS
'Verano'	865	48.1	NS	6.7	*	56.1	NS
PR1165-3	806	43.0	NS	7.2	**	47.5	NS
'Benítez'	741	41.4	NS	5.9	NS	49.6	NS
'Paraisito Mej. 2'	642	38.0	NS	6.3	**		
SEF 16	635	46.4	NS	6.9	**	31.8	NS
'ICA Pijao'	623	45.3	NS	5.5	**	54.2	NS
'Lenca Precóz'	583	45.5	NS	6.3	**	27.3	*

## Discussion

Soil and climatic conditions were suitable in this study to identify Mesoamerican bean lines having superior adaptation to low soil fertility and moderate levels of heat tolerance. Daytime temperatures >30 °C and nighttime lows >20 °C can reduce bean seed yield (Beebe et al., 2012). On the northern coast of Puerto Rico, from the months of June to October, mean daytime maximum temperatures normally range from 30-31 °C and nighttime minimum temperatures are often >25 °C (National Centers for Environmental Information, 2020). Therefore, lines in the four

**Table 7.** Least square means of carbon isotope discrimination of elite bean (*Phaseolus vulgaris* L.) lines from the Bean Abiotic Stress Evaluation panel (BASE 120), evaluated at Isabela, Puerto Rico, over three growing seasons. 2016-2018.**Tabla 7.** Medias ajustadas de discriminación de isótopos de carbono de líneas elite de frijol (*Phaseolus vulgaris* L.) del Panel de Evaluación de Estreses Abióticos del Frijol (BASE 120), evaluadas en Isabela, Puerto Rico, durante tres fechas de siembra. 2016-2018.

Genotype	Entry	June 2016	Rank	June 2017	Rank	June 2018	Rank
B12724	6	22.4	22	22.3	22	20.5	23
BAT 477	10	21.8	15	21.6	15	18.8	7
'Benítez'	12	21.6	8	21.5	8	19.5	18
'CENTA Pipil'	30	21.0	3	20.3	3	19.3	12
FBN 1203-43	36	21.1	4	20.8	4	19.3	13
FBN 1203-47	37	21.6	9	21.0	9	18.9	8
'ICA Pijao'	43	21.6	10	21.5	10	19.4	16
'INTA Centro Sur'	44	21.6	11	21.5	11	19.3	14
'Lenca Precoz'	53	21.7	13	21.2	13	20.1	19
MER 2212-28	54	21.1	5	20.9	5	19.0	9
MHN 322-49	55	20.8	2	21.4	2	19.1	10
PR1165-3	68	22.1	20	21.4	20	20.1	21
'Bella'	70	21.9	16	21.7	16	19.3	15
PR1418-15	71	22.0	19	21.1	19	19.4	17
PR1483-105	72	21.9	17	21.6	17	20.1	22
SEF 16	92	21.7	14	21.6	14	18.8	6
SER 118	98	21.6	12	21.1	12	18.4	3
'Rojo Chortí'	102	20.7	1	21.1	1	17.7	1
SB2-170	109	21.9	18	20.6	18	19.1	11
TARS-LFR1	110	21.5	7	21.3	7	17.9	2
TARS-MST1	111	22.4	23	21.4	23	18.7	5
'Verano'	117	21.4	6	21.4	6	18.7	4
'Sankara'	118	22.3	21	21.1	21	20.1	20

BASE 120 trials planted in June were exposed to moderate levels of heat stress throughout the growing season. Given the projections for climate change in Central America and the Caribbean, it would be appropriate for bean breeders in the region to routinely screen bean lines under warmer than currently normal temperatures.

The oxisol at the Isabela Substation has proven to be a useful site for screening beans for adaptation to low soil fertility (Dorcinvil et al., 2010). In four of the five BASE 120 trials soil nitrate levels were sub-optimum. In Colorado, USA soils having nitrate levels <11 are recommended to be fertilized for dry bean production at a rate of 67.25 kg ha<sup>-1</sup> of N (Davis & Brick, 2009).

Indirect selection for increased symbiotic nitrogen fixation can be successful on low N soils that are not fertilized (Bliss, 1993). Bean breeders in Central America and the Caribbean should regularly evaluate the performance of bean lines in unfertilized field trials.

Seed yield from the most promising lines from the BASE 120 trial were as great or greater than the mean seed of beans in Central America and the Caribbean (Food and Agriculture Organization, 2019). Several of the lines with superior performance in the unfertilized trials planted at Isabela, Puerto Rico possess additional traits of economic importance. SB2-170, a line that combined superior seed yield and non-significant GxE across environments, was selected for broad adaptation through a shuttle breeding program between the USDA-ARS in Puerto Rico and the University of Nebraska (McClellan et al., 2011). Another line that combined a mean seed yield >1,000 kg ha<sup>-1</sup> and

non-significant G×E across growing seasons was the small red TARS-LFR1. This line was selected in Puerto Rico for resistance to root rot and common bacterial blight, and for SNF and adaptation to low fertility soils (Porch et al., 2014).

In soils that are deficient in N, root growth and development, and SNF are favored to expand the capacity to acquire nutrients (Oldroyd & Leyser, 2020). The ability of Hermosa to produce superior seed yield, while having poor nodulation scores and low %NDFa, suggests that this black bean cultivar has the capacity to better utilize available soil nutrients. Beans can use different mechanisms to improve acquisition and efficiency of use of soil N (Dorcivil et al., 2010). Increased efficiency of nutrient acquisition or use would contribute sustainably to increased bean seed yield (Beebe et al., 2012).

Mean BRGA of the lines in this study were intermediate in magnitude. Soybean [*Glycine max* L. (Merr.)] plants with roots having intermediate (40-60 %) BRGA were reported to be well-suited for acquiring both soil water and nutrients (Zhao, 2004).

SNF is sensitive to abiotic stresses including drought, low soil fertility and high temperatures (Beebe et al., 2012). However, differences between bean genotypes in their SNF response to drought (Devi et al., 2013) and higher temperature (Fernández-Toledo et al., 1997) have been reported. Four of the five highest-yielding bean lines had three-year averages of %NDFa > 50 % which is greater than estimates of %NDFa for common bean in most trials conducted in different regions of the world where this grain legume is produced (Peoples, 2009). One study reported a mean %NDFa of 45.5 % in a subset of 259 lines of the Andean Diversity Panel evaluated in Michigan, USA (Kamfwa et al., 2015). In Ontario Canada, Wilker et al. (2019) obtained a mean %NDFa among Middle American bean lines to be 62.2 %. However, daylengths are longer, nights are cooler and net Fs may be greater at higher latitudes, which may favor SNF.

Pedigrees of the BASE 120 lines show that a large amount of genetic diversity was used to develop the elite Mesoamerican race bean lines in this study. It was possible to identify lines in this study that combined disease resistance with superior SNF. Breeding lines and cultivars that combine superior symbiotic nitrogen fixation and resistance to diseases including anthracnosis, common blight and BCMV have been identified (Wilker et al., 2019).

Although some of the elite lines in this study did not perform well in the low N soil at Isabela, Puerto Rico, they possess traits that may be useful as parents to develop cultivars for CAC. For example, the small red line SER 118 has yielded well and expressed greater yield efficiency in soils in Colombia having moderate levels of low P stress (Beebe et al., 2008) and terminal drought (Chaves-Barrantes et al., 2018). SEF 16 has resistance to BGYMV and has performed well in terminal drought and greater than optimum night temperatures (Chaves-Barrantes, 2015).

Carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) provides an indirect measure of water use efficiency in bean plants (Beebe et al., 2013). There were significant differences for  $\Delta^{13}\text{C}$  among entries and season × entry least square means were significant for all genotype × season combinations. Reduced SNF under drought associated with lower water use efficiency has been reported (Fenta et al., 2020). ‘Rojo Chortí’ was released in El Salvador (Parada-Cardona et al., 2015) and in Honduras (Rosas et al., 2019) as a heat and drought tolerant small red bean cultivar. Greater water use efficiency was associated with bean genotypes having lower  $\Delta^{13}\text{C}$  values and greater harvest index (Sanz-Saez et al., 2019). The  $\Delta^{13}\text{C}$  values reported on Mesoamerican beans planted in field trials in Ontario, Canada in 2011 and 2012 were greater than the  $\Delta^{13}\text{C}$  values in this study (Farid & Navabi, 2015). The lowest  $\Delta^{13}\text{C}$  estimate was 24.58 in Canada and the highest  $\Delta^{13}\text{C}$  value in this study was 22.4. Except for one location, where a negative association between  $\Delta^{13}\text{C}$  and %NDFa has been reported (Farid & Navabi, 2015).

This study identified elite Mesoamerican bean lines that possess unique combinations of traits that could be recombined in a breeding program to produce cultivars with superior ability to cope with biotic and abiotic constraints facing bean producers in Central America and the Caribbean. For example, the white bean cultivar ‘Bella’ had the highest overall seed yield and resistance to BGYMV, BCMNV, and common bacterial blight (Beaver et al., 2008). ‘Bella’ also produced more nodules and had estimates of %NDFa >50 %. The small red cultivar

‘Rojo Chortí’ combines BGYMV and BCMV resistance and tolerance to heat. Its low  $\Delta^{13}\text{C}$  suggests that ‘Rojo Chortí’ may also have characteristics that favor better adaptation to drought. Plants possess different mechanisms to adapt to soils deficient in nutrients (Oldroyd & Leyser, 2020). The black bean line ‘Hermosa’ produced superior seed yield despite having poor nodulation and a low estimated %NDFA. This line may possess characteristics that contribute to more efficient acquisition or utilization of available soil nutrients.

Lines of diverse genetic background were identified in this study that had superior SNF, thus additional cycles of recombination and selection may produce progenies with improved SNF characteristics. Using parents having different traits that were expected to contribute to symbiotic nitrogen fixation, recurrent selection in a controlled environment for increased nodule number of common bean lines was successful (Pereira et al., 1993). Recurrent selection to increase seed yield and shoot and seed N accumulation in a low N soil in Minnesota was also effective (Elisondo-Barron et al., 1999). Much of the progress was attributed to enhanced SNF. The authors recommended advancing lines to at least the  $F_4$  generation to increase the frequency of lines having desirable combinations of traits and to allow evaluation in replicated trials. Most breeding programs in Central America and the Caribbean have low N sites that could be used to routinely screen bean advanced generation breeding lines for greater SNF. Another study reported that both shoot dry weight and shoot N and seed yield and seed N have been reported to be highly correlated ( $r > 0.9$ ) (Elisondo-Barron et al., 1999). Therefore, evaluating bean lines for seed yield, biomass and days to maturity in a low N soil may provide an adequate preliminary screening for SNF. Indirect measures of biomass production using canopy reflectance indices (Gutiérrez-Rodríguez et al., 2006) would facilitate the screening of bean lines for this trait.

## Conclusions

This study identified elite Mesoamerican bean lines from the Bean Abiotic Stress Evaluation (BASE 120) panel with superior performance and enhanced symbiotic nitrogen fixation (SNF) in an unfertilized soil at Isabela, Puerto Rico. The pedigrees show that a wide array of genetic diversity was used to develop the elite lines in the BASE 120 panel and that many of these lines possess resistances to several of the most important biotic and abiotic factors limiting bean seed yield in Central America and the Caribbean (CAC). Bean breeders in CAC should be able to make use of this genetic diversity to continue to make significant progress developing improved Mesoamerican beans cultivars.

The occasional assembly, distribution and evaluation of regional nurseries containing elite Mesoamerican bean lines from different breeding programs could represent a valuable resource for bean breeding programs in Central America and the Caribbean. A regional nursery could also include the best sources of resistance to major biotic and abiotic constraints from other races and gene pools of the common bean. As technologies such as the molecular SNP assays become more accessible and cost-effective, Mesoamerican bean breeding programs should be able to utilize marker-assisted selection to introgressive genes of economic importance into Mesoamerican bean breeding lines.

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