AGRONOMIC PERFORMANCE AND GENETIC DIVERSITY OF COMMON BEAN (PHASEOLUS VULGARIS) VARIETIES IN HAITI

By

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To my husband, family and mentors

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TABLE OF CONTENTS

page

CHAPTER

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
AREA	Appui a la Recherche et au Développement Agricole (Support to Agricultural Research and Development)
ARS	Agricultural Research Service
BCMV	Bean Common Mosaic Virus
BCMNV	Bean Common Mosaic Necrosis Virus
BGMV	Bean Golden Mosaic Virus
BGYMV	Bean Golden Yellow Mosaic Virus
BL	Breeding Lines
GBS	Genotyping by Sequencing
CC	Cultivar Check
CIAT	International Center for Tropical Agriculture
FAMV	Faculty of Agronomy and Veterinary Medicine
IFAS	Institute of Food and Agricultural Sciences
LAI	Leaf Area Index
LBP	Legume Breeding Program
NGS	Next Generation Sequencing
ORE	Organization for Rehabilitation of the Environment
PCA	Principal Component Analysis
RAPD	Random Amplified Polymorphic DNA
SNP	Single Nucleotide Polymorphism
SNS	National Seed Service
SRB	Sample Resuspension Buffer
TREC	Tropical Research and Education Center

UF	University of Florida
UPGMA	Unweighted Pair Group Method with Arithmetic mean
UPR	University of Puerto Rico
USAID	United States Agency for International Development
USDA	United States Department of Agriculture

Abstract of Thesis Presented to the Graduate School
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Common bean (*Phaseolus vulgaris* L.) is one of the essential food legumes in the world and plays a vital role in providing nutritional security and revenue for low-income families in the Caribbean, including Haiti. Despite its economic importance, bean production in Haiti is constrained by many biotic and abiotic factors that limit yield. Besides, most bean cultivars currently in the market are low-yielding, averaging 0.6 tons/ hectare, which is well below the world average (0.86 tons/ ha). The current study aimed to evaluate the agronomic performance of 13 elite dry bean-breeding lines currently under development by the USAID-AREA and the Legume Innovation Lab programs at a highland location in Haiti. Moreover, the study aimed to determine the genetic diversity among a collection of 92 Haitian's bean cultivars using genotyping by sequencing. Analysis of variance and trait mean separation was done in R statistical package, while a UPGMA dendrogram based on genetic dissimilarity matrix was constructed using Darwin software. Significant differences were observed for most of the recorded parameters. The yield of bean varieties ranged from 0.48 to 1.24 tons/ha, with a mean of 0.89 t/ha. Among the traits measured, the number of pods per plant and number of seeds per pod showed the highest correlation with dry seed yield, thus may

be used for indirect selection of seed yield in common bean. Genetic diversity analysis revealed a prevalence of Mesoamerican gene pool within the accession collection, with a few of Andean origin, and some level of admixture.

CHAPTER 1
INTRODUCTION

Common bean (*Phaseolus vulgaris*) is a highly nutritive legume crop consumed by millions of people worldwide as a significant source of protein (Beebe et al., 2000). Other economically important Phaseolus species include *P. lunatus* (lima bean), *P. coccineus* (runner bean) and *P. acutifolius* (tepary bean). Phaseolus legume crops belong to Fabaceae family (Yuste-Lisbona et al., 2014) and are cultivated for their edible seeds and pods or unripe fruit (Beebe et al., 2013). They share this family with other important crops such as peanut (*Arachis hypogaea*), soybean (*Glycine max*), broad bean (*Vicia faba*), lentil (*Lens culinaris*), sweet pea (*Lathyrus odoratus*), cowpea (*Vigna unguiculata*), chickpea (*Cicer arietinum*) and alfalfa (*Medicago sativa*). *Phaseolus vulgaris* originated from Central America and was first cultivated in Central Mexico. The Mesoamerican populations were migrated to South America through different migration events (Bitocchi et al., 2012). Currently, it constitutes a significant food crop in the tropical, subtropical, and temperate regions of Africa, Europe, Asia, and the Americas (Wortmann, 2006). In 2017, the global area planted with bean was estimated at more than 36.4 million hectares, with a total production of 31.4 million tons (FAOSTAT, 2019). This crop is primarily produced in Latin America and Eastern and Southern Africa, where it is critical to nutritional security and income generation (Raatz et al, 2019).

Many biotic and abiotic factors limit common bean production in Haiti. Biotic factors include viral, fungal, and bacterial diseases, as well as insect pests, while abiotic factors include mineral toxicity, drought stress, high temperature, flooding, and nutritional deficiencies. Over the last two decades, collaborative breeding efforts have led to the recent release of superior disease resistant and early maturing bean varieties such as PR1146-138 and XRAV-40-4 in Haiti (Beaver et al., 2014, 2016). Nevertheless, the common bean cultivars in Haiti are still low-yielding, averaging 0.6 tons per ha in 2017. Also, there is a lack of knowledge on the genetic diversity of common bean varieties grown in Haiti, information that is necessary for breeding and conservation efforts.

Significance of the Study

Common bean is the most important source of protein in Haiti, especially for the resource-poor. Due to its economic and nutrition value, current breeding efforts are generated towards addressing production challenges in Haiti, including poor yield, biotic, and abiotic pressure. Towards this end, many initiatives have led to the release of improved cultivars exhibiting resilience to pests and diseases. However, more cultivars showing wide adaptability in various agroecological zones of the country are needed. Furthermore, knowledge of the genetic diversity of common bean accessions grown across the country is lacking. Therefore, the goal of the current study was to evaluate the agronomic performance of 13 elite common bean-breeding lines currently under development by the UF-AREA and the Legume Innovation Lab programs and determine the genetic diversity among 92 common bean accessions in Haiti. This project will aid the selection of advanced breeding lines for release to growers and elucidate the genetic structure of common bean accessions grown in Haiti for breeding and conservation purposes.

Objectives

This research project has two main objectives: i) determine the agronomic performance of 13 advanced breeding lines of common beans currently under development by the UF-AREA and the Legume Innovation Lab programs; and ii) determine the genetic diversity among 92 bean genotypes collected from different agro-ecological zones across Haiti using genotyping by sequencing.

CHAPTER 2
LITERATURE REVIEW

Environmental Adaptation

Common bean is a widely adaptable, warm-season herbaceous plant with distribution in tropical, subtropical, and temperate environments (Heuzé et al. 2015). The crop can grow within a wide altitude range from the sea level up to 2200 to 3000 m. The optimum annual rainfall required for the crop is between 500 and 1500 mm but can grow within a wide precipitation range (300 to 4300 mm). Common bean can tolerate environmental temperature up to 35 ^{o}C but prefers a range between 15 ^{o}C and 23 ^{o}C (Wortmann 2006; Heuzé et al. 2015). It can be cultivated in diverse types of soils, but ideally in well-drained silt loam, sandy loam or clay loam soils with high organic content and a pH between 4 and 9 (Ecoport, 2013). Mineral deficiencies may occur in acidic sandy soils (Mo and Mg) and calcareous Soils (Zn) (Heuzé et al., 2015; Ecoport, 2013; Wortmann, 2006). Despite its adaptation to diverse growing conditions, common bean shows sensitivity to certain elements such as B, Mn, Al, and high levels of Na.

The life cycle of the determinate common bean varies between 60 to 90 days, whereas that of the indeterminate climbing types can extend up to 300 days (Heuzé et al. 2015). The yield of common bean varies depending on the varieties, snap bean or dry beans. For snap bean varieties, where the green pod is harvested at about 25 to 30 days after flowering, the yield obtained can vary between 5 to 7.5 tons/ha. On the other hand, the average yield of dry beans is estimated at 0.5 to 1.5 tons/ ha, but higher yields up to 2.8-5 tons/ ha have been reported (Heuzé et al., 2015; Ecoport, 2013; Wortmann, 2006).

Nutritional Value of Common Bean

Common bean is rich in protein (22-27% dry matter), as well as starch (39-47% dry matter) (Heuzé et al., 2015). It is considered a valuable source of essential vitamins and minerals (iron and zinc), soluble fiber, and is of low-fat content (Zargar et al., 2014, Keller et al., 2015). Furthermore, beans contain a lot of bioactive compounds, including enzyme inhibitors, phytates, lectins, oligosaccharides, and diverse phenolic substances (Zargar et al., 2014). It has been observed that the presence of polyphenolic compounds in common bean reduces the risk for several disorders, including cardiovascular disease, diabetes, colon cancer, and obesity (Zargar et al., 2017). Based on their high content in vitamins and minerals, beans are often called the “poor man’s meat” in nutritional terms (Zargar et al., 2014).

Bean Production in Haiti

Common bean has been domesticated for over 8000 years from a wild vining plant distributed in the highlands of Middle America (Mesoamerican) and the Andes, to a significant leguminous food crop, widely adapted to a wide range of environments across the world (Gómez et al., 2004). In the Caribbean, common bean was introduced during the pre-Columbian era through trade by Taino and Arawak tribes from the Mesoamerican and Andean centers of origin (COG), respectively (Gepts et al., 1988). Since then, common bean has become the most important source of protein in the Caribbean, including Haiti, especially for the resource-poor who cannot afford animal-derived protein foods. The demand for common beans in Haiti has increased exponentially over the last half-century has demonstrated by a dramatic increase in production between 1961 (89,000 ha; 37,500 tons) and 2017 (171,850 ha; 111,398 tons) (FAOSTAT, 2019). However, within the same period, there was little increase in yield (0.42 tons ha-1 in 1961 to 0.65 tons ha-1 in 2017) (FAOSTAT, 2019), meaning that most of the observed shift in production was due to increased acreage. The current common bean production in Haiti is below the world average (0.86 tons ha-1 in 2017) (FAOSTAT, 2019).

In Haiti, common bean is grown in two main agro-ecological zones; lowlands and highlands (Colbert, 2017). The most consumed seed types in Haiti are black, red mottled, yellow, white, and pinto (Beaver et al., 2012). The locally produced beans are preferred over the imported bean. Common bean is grown in diverse areas across all departments of Haiti, but most predominantly in the West region followed by Artibonite and Center regions (Fig 2-1). The UF-AREA bean-breeding program conducts trials across locations in the West Department, including Duvier a highland site with an altitude of 887m and Cabaret a lowland location with an elevation of 51m.

Production Constraints in Haiti

Common bean production is affected by both biotic and abiotic factors wherever it is grown. According to Hnatuszko-Konka et al. (2014), beans yield is limited by six major diseases and several hostile abiotic conditions. In the Caribbean, diseases limiting bean yield include those caused by viral (Bean Golden Yellow Mosaic Virus, Bean Common Mosaic Virus, Bean Common Mosaic Necrosis Virus), fungal (e.g. rust caused by *Uromyces appendiculatus*, web blight caused by *Thanatephorus cucumeris*, and root and stem rots caused by *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina*), and bacterial (bacterial blight caused by *Xanthomonas axonopodis pv. Phaseoli*) (Beaver et al., 2012) pathogens. The major insect pests include leafhoppers (*Empoasca kraemeri*), leaf beetles (*Cerotoma* spp.), lepidoptera

(*Hedylepta indicata* and *Etiella zinckenella*) and bruchids (*Acanthoscelides obtectus* and *Zabrotes subfasciatus*) (Beaver et al., 2012).

Abiotic stress factors limiting common bean production in Haiti include mineral toxicity (e.g. B, Mn, and Al), drought and heat stress, flooding, low pH and poor soil fertility/ nutritional deficiencies (Colbert, 2017). In most cases, growers are unable to correct for these factors due to lack of awareness/ education, as well as lack of capital to buy agricultural inputs.

Natural disasters such as earthquakes and hurricanes also harm bean production in Haiti, but losses incurred vary from year to year. Between 2000 and 2017, about eight significant hurricanes have hit Haiti during bean growing seasons, causing devastating crop loss and field abandonment due to population displacements. Besides, socio-economic factors such as high cost of inputs and inaccessibility to the local market (e.g. poor infrastructure/ roads) negatively affect the value of bean production in Haiti.

Bean Breeding in Haiti

Due to the agronomic and economic importance of common bean in Haiti and around the world, several international collaborative initiatives have been undertaken to mitigate production challenges. Through the Medicago Genome Consortium and the International Conferences on Legume Genomics and Genetics, legume scientists discuss, prioritize and harmonize efforts in legume genomics and genetics to provide pragmatic solutions (Hnatuszko-Konka et al., 2014). In this context, an International consortium aimed to establish the best framework for advancing knowledge on bean was established in 2000 in Sevilla, Spain. Bean researchers at the Escuela Agricola Pan Americana (Zamorano), the Universities of Puerto Rico and Nebraska, and the

USDA-ARS have collaborated with CIAT, the Instituto Dominicano de Investigaciones Agropecuarias y Forestales and the National Seed Service of the Ministry of Agriculture of the Republic of Haiti to develop and release bean cultivars and improved germplasm for the Caribbean with the support from the Bean/Cowpea CRSP, the Dry Grain Pulse CRSP and recently the USAID-AREA and the Legume Innovation Lab programs (Beaver et al., 2012; Colbert et al., 2017). The primary focus of this great collaborative effort has been the development of high yielding, disease, and insect resistant cultivars adapted to the wide environmental variation in Haiti. Through these efforts, several bean cultivars have been developed and released to growers across the Caribbean, including Haiti. A non-exhaustive list of these cultivars is provided in Table 2-1.

Genetic Diversity of Common Bean

According to Schmutz et al. (2014), common beans are diploids organism (2n = 22) with a genome size of about 587 Mb. The small genome, coupled with a low index of genome duplication (most loci are a single copy), makes common bean a suitable experimental organism (Müller et al., 2014; Vidak et al., 2017). The recent availability of a draft genome for common bean has created opportunities for further inquiry into the genetic mechanisms underlying economically important traits such as yield and resistance to biotic and abiotic stress (Ariani et al., 2016; Meziadi et al., 2016; Schmutz et al. 2014).

Phenotypic and molecular markers have been extensively used for analysis of genetic diversity in common bean (Miklas et al., 2006; Kwak and Gepts, 2009; Blair et al., 2009), and have shown evidence of 2 major gene pools, Andean and Mesoamerican, which were domesticated independently (Kwak and Gepts, 2009; Bitocchi et al., 2012; Schmutz et al., 2014; Ariani et al., 2016). The Mesoamerican gene pool is adapted to lower altitudes and higher temperatures than the Andean gene pool (Ariani et al., 2016). The gene pools are further divided into six races according to morphological criteria and agro-ecological adaptation, three each for Mesoamerican (Durango, Jalisco, and Mesoamerica) and Andean (Chile, Nueva Granada and Peru) (Blair et al., 2009).

Several authors have examined genetic diversity among common bean landraces in Central America and Caribbean regions. Gomez et al. (2004) evaluated the pattern of genetic diversity among nine red-seeded landraces from Nicaragua with molecular and phenotypic markers and found that variation uncovered at the molecular level were due to the difference among and within landraces, while differences at the phenotypic level were attributed to adaptation to agro-ecological zones. For each landrace, twelve individuals were genotyped with seven bean microsatellite markers, while fourteen phenotypic traits were measured in two zones. These results implied that molecular differentiation was due to a founder effect, whereas the phenotypic variation was due to the effect of adaptation. While investigating the genetic diversity among 65 common bean landraces in the Caribbean using morphological and molecular markers, Durán et al. (2005) found that the accessions could be grouped into Mesoamerican and Andean gene pools. Mesoamerican phenotypes comprised all the red mottle lines from Haiti and three landraces from the Dominican Republic collected near the Haitian border, while Andean phenotypes consisted of lines from Puerto Rico and the Dominican Republic. Blair and Lorigados (2016) evaluated the diversity among 210 common bean landraces in Cuba using 36 SSR markers and found the majority of the genotypes to be Mesoamerican, with a few of Andean origin. The level of introgression between the two gene pools was lower than that previously observed in germplasm from other secondary centers of diversity, thus implying that Cuban beans are most likely derived from race Mesoamerica and race Nueva Granada, with a little mixing from other races.

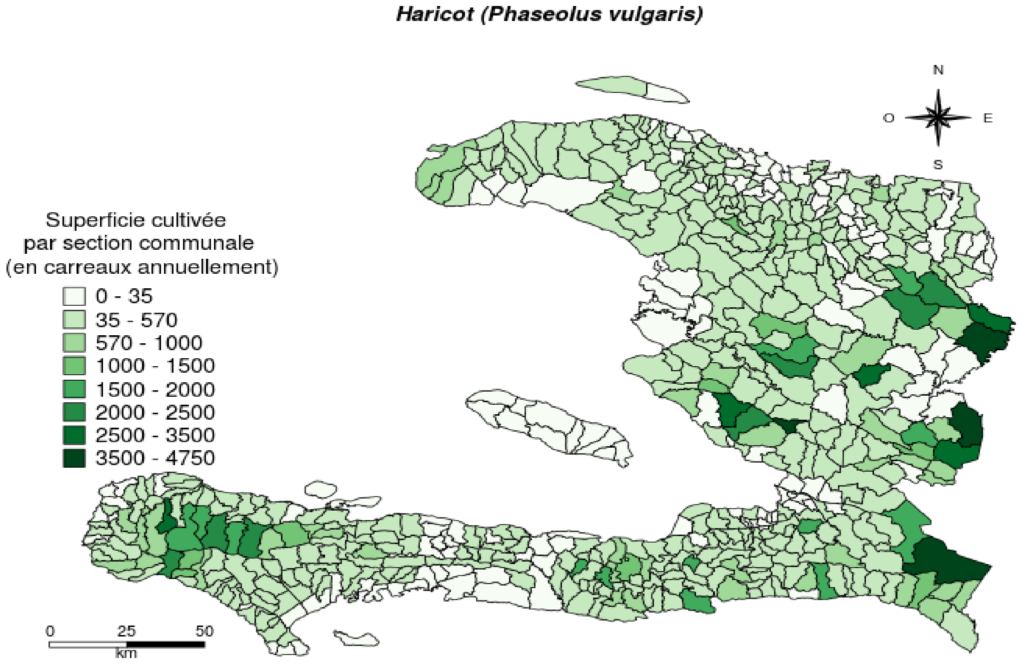
Beyond the Caribbean, genetic diversity among common bean landraces has also been reported. For example, using morphological and microsatellite markers, Asfaw et al. (2009) evaluated the genetic diversity and population structure among 192 common bean landraces from East African (Ethiopia and Kenya) highlands. The study revealed considerable genetic and phenotypic diversity that corresponded to the two recognized gene pools (Andean and Mesoamerican) with little introgression between these groups. Moreover, it was observed that the genetic divergence was slightly higher for the Ethiopian landraces compared to Kenyan landraces and that Mesoamerican genotypes were more diverse than the Andean genotypes. Becerra et al. (2010) characterized 237 Chilean common bean landraces using microsatellite markers and found that Andean genotypes were predominant. Race Chile was found closely related to races Nueva Granada and Peru of the Andean gene pool, but further differentiated from the race Mesoamerica of Mesoamerican gene pool. The substantial genetic differences between the two races (Chile and Mesoamerica) suggested the potential value for novel allele transfer between the two gene pools, although it may be challenging to combine genetic crosses due to hybridization barriers. Maras et al. (2015) reported high genetic diversity among 119 common bean landraces in the Western Balkans using 13 SSR markers where Andean genotypes were more prevalent. In 2018, Campa et al. conducted a molecular characterization of a Spanish diversity panel of 308 common bean lines through Genotyping-by-Sequencing. The panel was characterized by 3,099 single-nucleotide polymorphisms, which revealed a wide genetic variation and a low level of redundant material within the Spanish bean panel. The two main gene pools were identified through Structure, cluster, and principal component analysis. However, most of the lines (70%) were associated with the Andean gene pool. Furthermore, lines showing intergene pool introgression were also observed, which suggest the use of the two gene pools in the breeding of snap bean cultivars. Accessions with similar genetic profile were identified, which may need to be removed to maximize the panel diversity. The usefulness of the panel for future GWAS was also validated through association mapping of determinacy.

Similarly, an in-depth genome characterization of a Brazilian common bean core collection was conducted by Valdisser et al. 2017, using DArTseq high-density SNP genotyping. In this study, 6,286 SNPs were genotyped in genic (43.3%) and non-genic regions (56.7%) which allowed the genetic subdivision based on the two main gene pools (K=2), and grain types (k=3 and k=5). A total of 83% of all SNPs were polymorphic in the Andean gene pool while 91% were polymorphic within the Mesoamerican gene pool, while 26% of all SNPs were able to distinguish the gene pools. The findings of this study showed that the DArTseq approach could generate a large set of useful SNPs for common bean with complete genome coverage, where both coding and non-coding regions are represented. This approach also allowed accurate evaluation of genetic diversity in the Brazilian beans collection.

An optimization of genotyping by sequencing (GBS) data in common bean was conducted by Schröder et al., 2016. The main goal of this project was to improve the quality and the coverage of GBS data in common bean (*Phaseolus vulgaris* L.) to increase the number of SNPs available for genome-wide association studies (GWAS). Twenty-five common bean genotypes from the Mesoamerican gene pool were used for comparison of 2 libraries by using the standard ApeK1 fragments and MseI/Taq α I double-digest fragments. The results revealed an increase of 3.8 to 12.5-fold in SNPs based on a minimum coverage (3X, 5X, and 8X).

Table 2-1. New bean varieties released in Haiti since 2006

Variety	Market type	Characteristics	Country/Institution collaboration	Author, year
PR1146-138	yellow	Resistance BGYMV (bgm), BCMV, BCMNV (bc-3)	UPR / SNS Haiti / USDA-ARS	Beaver et al., 2016
XRAV-40-4	black	Resistance BGYMV (bgm), BCMV, BCMNV (bc-3), earliness	UPR / UNL EEA/ USDA-ARS / IDIAF Rep Dom. /EAP Zamorano / SNS Haiti	Beaver et al., 2014
PR0633-10	Red mottled	Resistance BGYMV (bgm), BCMV, BCMNV (bc-3) (I)	UPR / USDA-ARS / IDIAF Rep. Dom. /SNS Haiti	Prophete et al., 2013
PR0737-1	Red mottled	Resistance BGYMV (bgm), BCMV, BCMNV (bc-3)	UPR / USDA-ARS / IDIAF Rep. Dom./SNS Haiti	Prophete et al., 2013
PR9745-232	Red mottled	Resistance to BGYMV (bgm) and BCMV (I)	CIAT / UPR /IDIAF Rep. Dom./ SNS Haiti	Blair et al., 2006
RMC-3	Red mottled	Resistance to BGYMV (bgm) and BCMV (I)	CIAT/ UPR/ IDIAF Rep. Dom./ SNS Haiti	Blair et al., 2006



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Sources : CNIGS, MARNDR (RGA 2009), IHSI (Atlas Censitaire 2003), © Contributeurs OpenStreetMap
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Figure 2-1. Bean planted area in Haiti

CHAPTER 3
AGRONOMIC PERFORMANCE OF COMMON BEAN VARIETIES IN HAITI

Background

Common bean is one of the most essential food legumes in the world and plays an important role in providing nutritional security and revenue for low-income families in the Caribbean countries, including Haiti. It is a main constituent of the daily diet in Haiti, where consumers primarily prefer black, red mottled, yellow, white and pinto bean (Beaver et al., 2012).

Despite its economic importance, bean production is constrained by many biotic stresses including viral, fungal and bacterial diseases, as well as insect pests. Abiotic factors limiting bean production include extreme pH, low soil fertility, salinity, drought and heat stress (Colbert, 2017). These factors, combined with the variable agro-ecological zones in the country, emphasize the need to develop widely adapted bean germplasm with acceptable biotic and abiotic resilience. Currently, most of the common bean cultivars grown in Haiti are low yielding, averaging 0.6 tons/ ha, a value significantly less than the world average 0.86 tons/ha.

Over the last two decades, collaborative breeding efforts have led to recent release of superior bean varieties, seven of which have been released since 2006 (PR1146-138, XRAV-40-4, PR0633-10, PR0737-1, AIFI Wuriti, PR9745 -232 and RMC-3) (Beaver et al., 2014; Beaver et al., 2016; Blair et al., 2006; Prophete et al., 2013; Rosas et al., 2008). These lines exhibit superior agronomic performance over traditional landraces for traits such as earliness (XRAV-40-4), tolerance to drought and low soil fertility (AIFI Wuriti), and improved resistance to viral diseases (BGYMV, BCMV and BCMNV). Currently, breeding efforts are underway to develop more high-yielding cultivars through a partnership between USAID-AREA project and the Legume Innovation Lab. The goal of this study was to evaluate the agronomic performance and phenotypic diversity among 13 advanced common bean breeding lines and identify the best lines for further selection and release to growers in Haiti.

Materials and Methods

Plant Materials

Thirteen advanced breeding lines from UF-AREA and the Legume Innovation Lab programs, as well as 12 cultivar checks, were used in the study. This germplasm pool consisted of twelve black, five red-mottled, one red, one pinto, one beige, and five white bean types (Table 3.1).

Experimental Site and Research Design

The experiment was conducted at Duvier, a highland location in the west region of Haiti (Figure 3.1). This trial was performed during the first bean cropping season of the year (April to July 2018). During this year, the experimental site received a monthly rainfall that ranged from 0.55 to 150.1 mm (Fig 3.2) and the temperature between 23 to 29 ^{o}C (Fig 3.3). The soil preparation was conducted using conventional tillage, and the bean lines were sowed manually in three replicated plots in a randomized complete block design. Each replicate or plot consisted of 62 individuals. For each plot, the genotypes were planted in two rows of 31 plants in a 3.6 m^2 area. In-row spacing was 10 cm, while between-row spacing was 60 cm and the distance between the block and the plots was one meter. The plots were maintained using hand weeding and basic pest management practices. Phenotypic assessment of bean lines was done by measuring eleven agronomic traits, according to Gomez et al., 2004 (Table 3.2). Besides, leaf area index, which corresponds to the ratio of leaf surface area over a total ground surface area, was determined by using LAI meter: AccuPAR LP-80. The diseases that were more prevalent during the experiment including Powdery Mildew, Bean Common Mosaic Virus (BCMV), Bean Golden Mosaic Virus (BGMV) were evaluated using a scale of 1 to 9 according to CIAT where 1 to 3 is resistant, 4 to 6 intermediate and 7 to 9 susceptible.

Data Analysis

All data collected were subjected to analysis of variance (ANOVA) using R-3.6.0. The assumptions required for linear models, including normality, independence of errors, and linearity were tested before running the ANOVA test. The three traits were considered as co-factors in the model to account for the influence of growth habit, gene pool, and disease incidence on seed yield. The least-square means multiple comparison test was used to identify significant differences between the genotypes for the different phenotypic traits assessed. Pearson correlation was performed for the phenotypic traits. Furthermore, the mean values of these traits were used to perform principal component analysis (PCA) to identify patterns of phenotypic variation.

Results

This project aimed to assess the agronomic performance of 13 advanced breeding lines of common bean along with 12 cultivar checks. However, two cultivar checks (Local-4 red and Local-6 Pinto) did not germinate well in the field. Thus, the results are presented for the remaining 23 genotypes.

Agronomic Traits

Analysis of variance and mean separation revealed significant differences ($p < 0.05$) among the genotypes for most of the traits evaluated (Table 3.3). The yield ranged from 0.48 to 1.24 tons ha-1. Overall, the Mesoamerican bean types (black, white, and beige) were significantly higher in yield, pods/ plant, seeds/pod than their Andean counterparts, except for seed weight (Figure 3-4). The three varieties that had highest yields included two cultivar checks, Verano and LORE 234 Local, and the breeding line PR1627-8. The genotypes showed a significant difference in the number of pods per plant with a range of 4.33 to 8.87. The number of seeds per pod ranged from 3.15 to 5.73 and differed significantly ($P<0.05$) among the bean cultivars. The value recorded for 100 seed weight showed statistically significant differences among the genotypes and ranged between 12.62 and 28.26 g (Table 3.3).

Phenological and Morphological Traits

The phenological traits assessed in this experiment included days to 50% flowering, days to 50% maturity, and earliness that was calculated based on the difference between day to 50% flowering and to 50% maturity. The accessions showed significant differences ($P<0.05$) for the three parameters (Table 3-4). The number of days to flowering ranged from 37 to 43 days, the number of days to maturity from 56 to 68 days and earliness ranged from 19 to 27 days after flowering. The earliest varieties included the breeding line PR1423-153 and two cultivar checks LORE 234 local, and local three red, all of which reached physiological maturity within 19 to 20 days from flowering (Fig 3-5). The accessions also showed significant differences for the morphological traits evaluated, including stem length, pod length, and pod width (Table 3-5). The value recorded for the stem length ranged from 25.30 cm to 38.67 cm. The pod length for the bean genotypes ranged from 8.02 to 11.77 cm, and the range for pod width varied from 1.00 to 1.31 cm. Leaf area index exhibited a significant difference among the genotypes and ranged from 1.13 to 2.65 (Fig 3-6).

Qualitative Traits

Three types of wing petal color were recorded for the genotypes in the experiment, including white, purple, and pink. From the 23 genotypes, 2 had pink flowers, 10 had white flowers, and 11 had purple flowers (Table 3-6). Based on the results, all the white beans had white flowers. However, white-colored flowers were also found for colored seeds, such as black and red seeded types in the current experiment.

The common bean genotypes used in the study had either determinate or indeterminate type II growth habit. However, the indeterminate type II was more prevalent, with 74% of the genotypes showing this growth habit (Table 3-6).

Evaluation of Common Bean Genotypes for Disease Resistance

The most prevalent diseases in the field were evaluated during the field trial. The diseases that were assessed include powdery mildew, Bean Golden Mosaic Virus (BGMV), and Bean Common Mosaic Virus (BCMV) using a scale of 1 to 9. The genotypes showed a significant difference in their response to different diseases (Table 3-7). Based on the scale of CIAT for evaluation of powdery mildew, 14 varieties were found resistant, eight varieties intermediate and one susceptible (Table 3-8). Significant differences were recorded in the response of the genotypes to viral diseases: BGMV and BCMV. Among the lines, 16 showed resistance to BGMV, while seven were intermediate for strength. In contrast to the other diseases, the plots were more affected by BCMV with only seven varieties showing resistance, 14 intermediate and two susceptible. On average, the white beans were less affected by both fungal and viral diseases evaluated in the field, followed by black beans (Fig 3-7).

Phenotypic Correlations

The Pearson correlation analysis between phenotypic traits is presented in Figure 3-8. Flowering time (days to flowering) was positively correlated with the number of days to 50% maturity, number of pods per plant, number of seeds per pod, yield, LAI, and growth habit, but it was not significantly correlated with earliness. A significant positive correlation was observed between days to 50% maturity and earliness. Seed yield was positively associated with the number of pods per plant, number of seeds per pod and growth habit, with the strongest correlation with the number of pods per plant. Seed weight was positively correlated with stem length, pod length and pod width, whereas it was negatively correlated with the number of seeds per pod, number of pods per plant, flowering time and growth habit. A positive correlation was recorded between leaf area index and 50% maturity, the number of pods per plant, stem length, and flowering time.

Principal Component Analysis

The total proportion explained by the 11 PCs and corresponding vector loadings are presented in Table 3-9. The first principal component (PC1) captured the maximum amount of variation (53%) and separated the genotypes mainly based on growth habit, seed weight, number of seeds per pod, number of pods per plant, stem length, pod length, and pod width. On the other hand, the second PC explains 17.44% of the variation and separated the varieties mainly on leaf area index, day to flowering, and maturity. The third PC captured about 13% of the variation and separated the genotypes based on yield, pods per plant, pod length. Together, PC1, PC2, and PC3 explained 83.29% of the total phenotypic variation. A PCA plot based on PC1 and PC2 revealed three major bean-type clusters (Fig 3-9). The red mottled accessions formed a distinct cluster and were characterized by high seed weight, pod length, and pod width. Black beans formed the largest cluster and varied widely in yield, pods per plant, seeds per pods, days to maturity, and leaf area index. On the other hand, the white beans formed a tight cluster that showed less variation for the traits assayed. The accessions of beige and red bean type were respectively clustered close to the Mesoamerican and Andean bean types.

Discussion

In the current study, the agronomic performance of 13 advanced breeding lines of common bean was evaluated in a highland location in Haiti (Duvier). Considerable variation was observed among the genotypes for most of the assessed parameters.

Overall, Mesoamerican cultivars outperformed Andean cultivars in all agronomic traits, except for seed weight. The difference in agronomic performance between the two gene pools is expected and has been widely reported in the literature (Singh et al., 1991a; White & Gonzáles 1990; White et al., 1992; Sexton et al., 1994). As expected, seed yield was highly influenced by growth habit and disease incidence. When these traits were considered as cofactors in the model, no significant differences were observed for yield across the genotypes. The yield was highly correlated with the number of pods per plant as well as the number of seeds per pod, indicating that these traits may be used for indirect selection of dry seed yield. Significant positive correlations between these traits have been previously reported for common bean (Mebrahtu et al., 1991). These observations are expected because the number of pods per plant and number of seeds per pod is the most critical components of seed yield in common bean (Ambachew et al., 2015; Mebrahtu et al., 2001). The relationship among the three traits may indicate pleiotropic or genetic linkage for the underlying loci (Dilday et al., 1990), thus allowing for tandem selection of the traits (Ambachew et al., 2015). Significant negative correlations were found between seed size (seed weight) and number of pods per plant (-0.31) and the number of seeds per pod (-0.59). This result confirms previous observation that yield difference between Andean and Mesoamerican gene pools is due to seed size (White and Gonzáles,1990).

The accessions differed significantly in phenological and morphological traits. Within the Mesoamerican gene pool, the cultivar LORE 234 Local (black bean) had the least flowering and maturity time, while within the Andean gene pool, Local3 Red was the best cultivar for the two traits. Therefore, the two cultivars may be used to improve earliness in the Haiti bean-breeding program. As expected, days to 50% flowering and days to 50% maturity were positively correlated (Zeven et al., 1999). However, the number of days to 50% flowering was not significantly correlated to earliness. This situation may be explained by variation in the growth habit of the accessions. Some bean accessions with indeterminate growth habit may take longer to mature, despite reaching 50% flowering relatively early compared to a determinate variety. Therefore, the number of days the plant takes to reach maturity after flowering is a better indicator of earliness than the number of days from sowing to maturity.

Similarly, day to flowering was positively correlated with the number of seeds per pod and number of pods per plant, further confirming the phenological distinction between the Andean and Mesoamerican gene pools. A positive and significant correlation was recorded between stem length, pod length, and pod width, with the highest correlation between pod length and pod width. Zeven et al. (1999) reported a positive correlation between pod length and pod width in common bean. A similar relationship was observed between pod length and width, and with seed weight, and may be explained by the fact that the larger seeded bean types which have higher seed weight require longer and larger pods to the contrary to the small-seeded types.

Similar to findings by Zeven et al. (1999), white bean accessions exclusively had white-flower color. On the contrary, white flowers were found across the other bean types. Indeterminate growth habit was observed in 74% of the genotypes (Mesoamerican), while the rest (26%; Andean) were determinate type. As with previous studies, growth habit was positively correlated with pods per plant and seeds per pod (Kornegay et al, 1992).

Significant differences in disease severity were observed among the cultivars. The breeding lines (PR1423-99, PR1423-110, PR1423-153, PR1564-20, PR1564-3, PR1564-53, PR1627-10 and PR1627-8) were resistant in their response to powdery mildew, thus may be used as the source of resistance in the breeding program. For BGMV, 9 of the 13 breeding lines were found resistant, and five were intermediate. The response of the breeding lines to BGMV could be explained by the presence of *bgm* gene (Table 3-1). As previously revealed by Urrea et al., 1994; Velez et al. 1, 998 and Blair et al., 2007, the *bgm* that is located on Pv03 confer resistance to chlorosis inducing of BGMV infection. Eleven of the breeding lines (74%) were found to have intermediate to high resistance to BCMV. This was expected because they carry *I* and *bc-3* genes. The *I* gene that has a nearly terminal position on Pv02 (Vallejos et al., 2000), and *bc-3* gene located on Pv06 (Johnson et al., 1997) are recognized to confer resistance to all known strains of BCMV and BCMNV in common bean (Mukeshimana et al., 2005). Two of the breeding lines were rated as susceptible to BCMV, indicating that the resistance alleles in these lines was not fixed, and may have been lost through repeated selfing in the breeding program. Since the current experiment was conducted in one location, it is necessary to confirm resistance responses across all the genotypes in multiple environments in the future.

As expected, the principal component analysis revealed distinct clustering according to bean type. The red mottled accessions formed a different cluster characterized by high seed weight, pod length and pod width, while black and white beans formed separated unique clusters.

In conclusion, the results reported in this study showed a significant difference among the genotypes for most of the traits measured. The PR1627-8 (white bean) outperformed the other breeding lines in yield and disease resistance; therefore, it is a candidate for release to growers. Furthermore, the cultivars checks, including LORE 234 local, and Local 3 red could be used as a source of earliness for breeders. Since the data used was only for one season and one location, further trials are necessary to validate the results. Based on phenotypic relationships, the number of pods per plant and number of seeds per pod may be useful traits for indirect selection for seed yield in common bean.

Limitations

The current project has its limitations, which may affect its reproducibility. This research was conducted under conditions typical for Haitian farmers (agriculture with low input). The goal was to select varieties that can thrive under different biotic and abiotic stress with minimal input. The field trial was rainfed with no fertilizer application. In addition, no pesticides were applied during the experiments, and the varieties differed in their response to the diseases assessed (powdery mildew, BCMV, and BGMV). All these cofounding factors ought to be considered when interpreting the results reported here. The growth habit of the different gene pools may also affect the yield of the genotypes. For instance, the Mesoamerican lines with their indeterminate growth habit usually have a higher yield than the Andean lines that have determinate growth habit. However, to reduce the effect of some of the cofounding factors, disease incidence, as well as growth habit, were included in the ANOVA model for yield.

Figure 3-1. Location of the experimental site

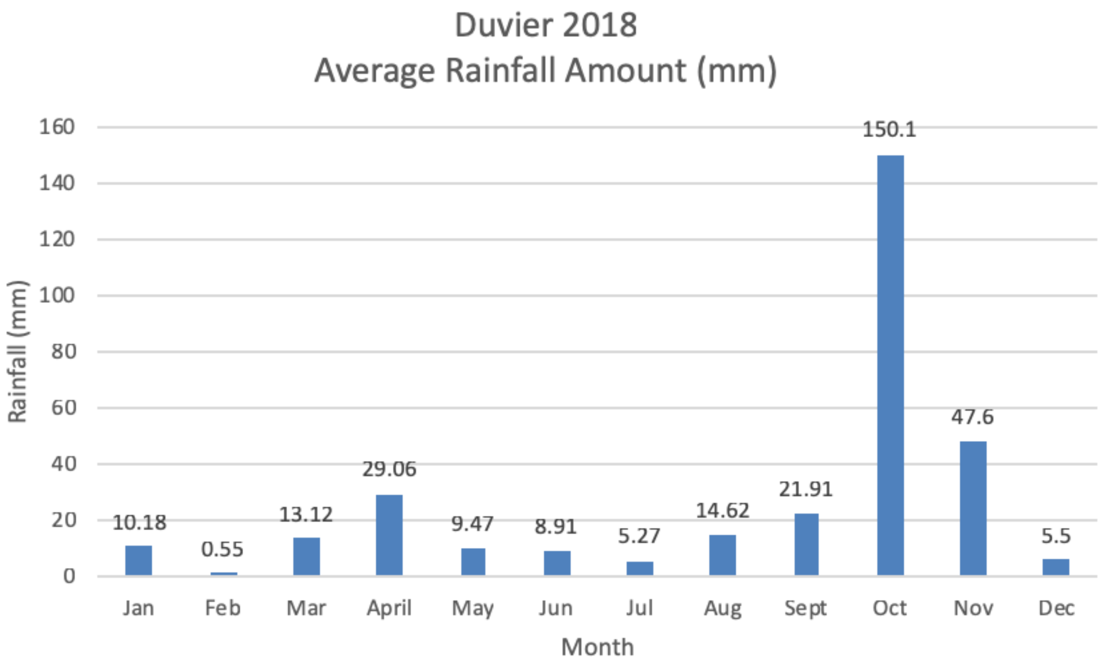

Figure 3-2. Rainfall of the experimental site

Data adapted from Worldweatheronline.com

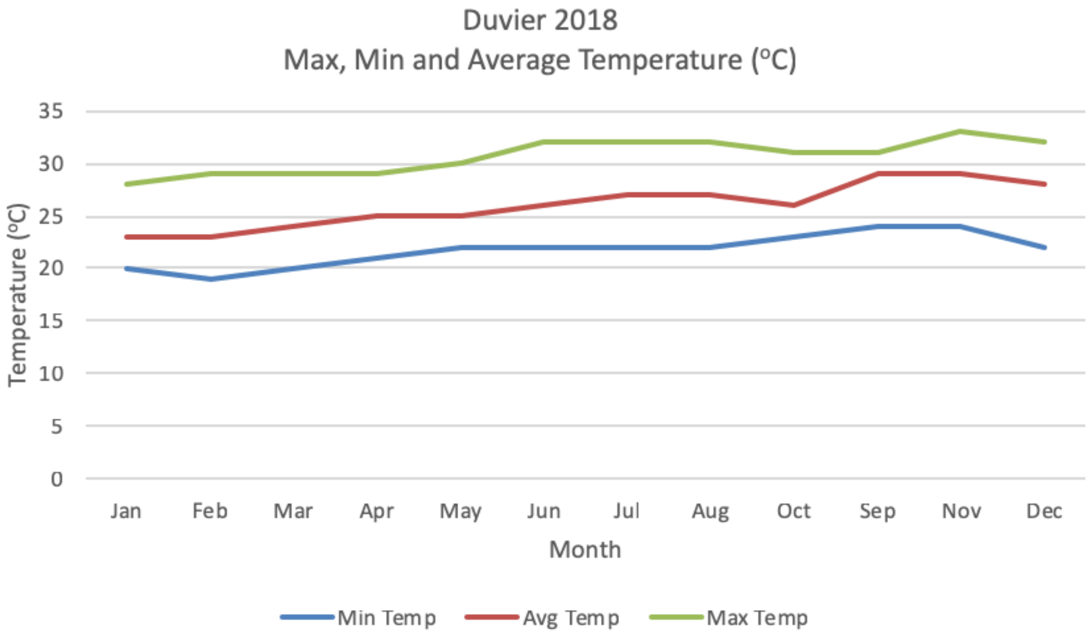

Figure 3-3. Temperature of the experimental site

Data adapted from Worldweatheronline.com

Table 3-1. Common bean accessions used in the study

Breeding line	Market type	Characteristics	Country/Institution collaboration
PR1423-99	Black	*bgm, I, bc3,* CBB	Univ. of Puerto Rico & UF-Haiti AREA-LBP
PR1423-110	Black	*bgm, I, bc3,* CBB	Univ. of Puerto Rico & UF-Haiti AREA-LBP
PR1423-117	Black	*bgm, I, bc3,* CBB	Univ. of Puerto Rico & UF-Haiti AREA-LBP
PR1423-153	Black	*bgm, I, bc3,* CBB	Univ. of Puerto Rico & UF-Haiti AREA-LBP
XRAV-40-4 (Sankara)	Black	*bgm, I, bc3,* CBB	Univ. of Puerto Rico, Univ. of Nebraska, USDA-ARS, Univ. Zamorano & National Seed Service Haiti
MEN2201-64	Black	*bgm, I, bc3,*	
PR1564-20	Black	*bgm, I, bc3,*	Univ. of Puerto Rico & UF-Haiti AREA-LBP
PR1564-53	Black	*bgm, I, bc3,*	Univ. of Puerto Rico & UF-Haiti AREA-LBP
PR1564-3	Black	*bgm, I, bc3,*	Univ. of Puerto Rico & UF-Haiti AREA-LBP
PR1627-8	White	*bgm, I, bc3,*	Univ. of Puerto Rico & UF-Haiti AREA-LBP
PR1627-10	White	*bgm, I, bc3,*	Univ. of Puerto Rico & UF-Haiti AREA-LBP
PR1627-13	White	*bgm, I, bc3,*	Univ. of Puerto Rico & UF-Haiti AREA-LBP
Verano	White	*bgm, I, bc3,*	
PR1654-1	Red Mottled	*bgm, I, bc3,*	Univ. of Puerto Rico & UF-Haiti AREA-LBP
PR1654-2	Red Mottled	*bgm, I, bc3,*	Univ. of Puerto Rico & UF-Haiti AREA-LBP
PR1654-3	Red Mottled	*bgm, I, bc3,*	Univ. of Puerto Rico & UF-Haiti AREA-LBP
Badillo	Red Mottled		
Salagnac-local	Black		
LORE 234 local	Black		ORE Haiti & UF-Haiti AREA-LBP
Local1	Red mottled		
Local2	Black		
Local3	Red		
Local4	White		
Local5	Beige		
Local6	Pinto		

Table 3-2. Phenological (P), morphological (M), qualitative (Q), and agronomic (A) traits measured in the study

Character	classification	Description
Day to flowering (DAP)	P	Number of days from sowing to the stage when 50% of the sampled plants have begun to flower
Physiological maturity (DAP)	P	Number of days from sowing until 50% of the sampled plants have changed the color of their pods
Stem length (cm)	M	The distance from the ground surface to the tip of the main guide at flowering. Sample size: 10 plants plot^{-1}
Pod length, cm	M	Exterior distance from the pod apex to the peduncle. Sample size: 30 pods plot^{-1}
Pod width, cm	M	Distance from the right angle to the sutures as the middle of the pod. Sample size: 30 pods plot^{-1}
Growth habit	Q	Determined according to Muñoz et al (1993). Sample size: 10 plants plot^{-1}
Wing petal color	Q	Determined in freshly opened flowers according to Muñoz et al (1993). Sample size: 10 plants plot-1
Pods plant^{-1}	A	Average number of fertile pods plant$^{-1.}$ Sample size: 10 plants plot^{-1}
Seeds pod^{-1}	A	Average number of seeds pod^{-1}. Sample size: 30 pods plot^{-1}
100 seed weight, g	A	Average 100-seed weight (14% moisture)
Yield per plot, kg ha^{-1}	A	Determined on the basis of the total number of harvested plants plot^{-1}

Table 3-3. Multiple comparison of means for agronomic traits of the genotypes

			Agronomic traits			
Lines	Market type	Varieties	Yield (tons/ha)	Pods/plant	Seeds/pod	100 seeds weight
CC	Be	Local5 Beige	0.83 ns	6.47abcd	4.67cdef	15.87ab
CC	Bl	Local2 Black	0.92 ns	6.03abcd	5.30def	16.93ab
CC	Bl	LORE 234 Local	1.24 ns	8.87d	4.92def	16.43ab
CC	Bl	MEN2201-64	0.96 ns	6.47abcd	4.98def	16.76ab
BL	Bl	PR1423-110	0.78 ns	6.10abcd	5.27def	14.11ab
BL	Bl	PR1423-117	0.76 ns	5.77abcd	5.25def	14.61ab
BL	Bl	PR1423-153	0.99 ns	6.93abcd	5.72f	14.54ab
BL	Bl	PR1423-99	0.88 ns	6.10abcd	5.73f	14.54ab
BL	Bl	PR1564-20	0.82 ns	7.20abcd	4.48cde	14.34ab
BL	Bl	PR1564-3	0.97 ns	7.90bcd	5.55ef	12.62a
BL	Bl	PR1564-53	1.02 ns	7.10abcd	5.38def	15.17ab
CC	Bl	Salagnac local	0.94 ns	6.97abcd	4.67bcd	16.57ab
CC	Bl	XRAV-40-4	0.78 ns	5.63abcd	5.00abc	15.96ab
CC	R	Local3 Red	0.88 ns	5.00ab	3.58abc	28.26ab
CC	R M	Badillo	1.01 ns	5.40abc	4.31bcd	23.55ab
CC	R M	Local1 RM	0.48 ns	4.33a	3.15a	18.96ab
BL	R M	PR1654-1	0.61 ns	5.67abcd	3.30ab	18.40ab
BL	R M	PR1654-2	0.66 ns	5.70abcd	3.33ab	19.55ab
BL	R M	PR1654-3	0.73 ns	4.80ab	3.35def	26.12cd
BL	W	PR1627-10	0.98 ns	7.57abcd	4.83def	15.63d
BL	W	PR1627-13	0.94 ns	7.47abcd	4.95def	14.77bc
BL	W	PR1627-8	1.16 ns	8.47cd	5.30def	15.10abc
CC	W	VERANO	1.16 ns	8.70cd	4.65cdef	15.90bc

CC: cultivar check, BL: breeding line, Be: beige, Bl: black, R: red, RM: red mottled; W: white; ns: no signifance

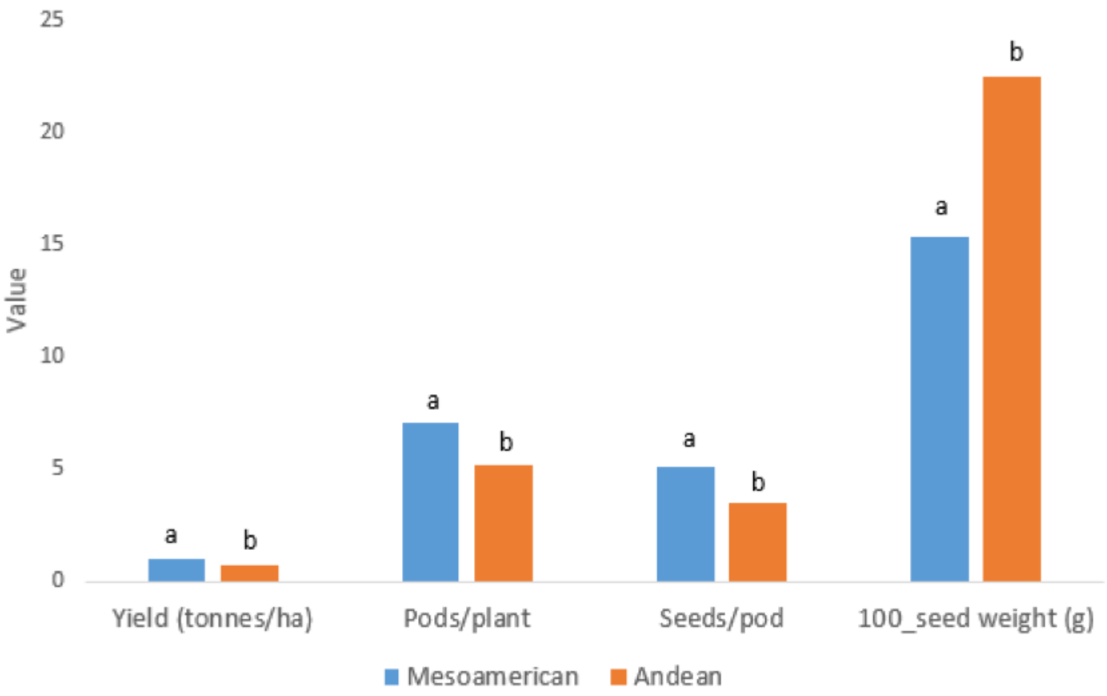

Figure 3-4. Variation in yield and yield components between cultivars of Mesoamerican and Andean gene pools in the current study

Table 3-4. Multiple comparison of means for the phenological traits of 23 genotypes

Lines	Bean type	Varieties	Phenological traits 50% flowering	 50%Maturity	 Earliness
CC	Be	Local5 Beige	39.33abcd	61.00abcd	21.67abc
CC	Bl	Local2 Black	43.00e	67.67g	24.67abc
CC	Bl	LORE 234 Local	37.00a	56.00a	19.00a
CC	Bl	MEN2201-64	37.33ab	59.00abc	21.67abc
BL	Bl	PR1423-110	41.00cde	61.67bcde	20.67ab
BL	Bl	PR1423-117	40.33abcde	61.00abcd	20.67ab
BL	Bl	PR1423-153	40.00abcde	59.00abc	19.00a
BL	Bl	PR1423-99	40.67bcde	65.67defg	25.00abc
BL	Bl	PR1564-20	41.00cde	65.00defg	24.00abc
BL	Bl	PR1564-3	42.33de	66.33fg	24.00abc
BL	Bl	PR1564-53	41.67de	68.00g	26.33bc
CC	Bl	Salagnac local	41.67de	62.00cdef	20.33ab
CC	Bl	XRAV-40-4	37.33ab	59.00abc	21.67abc
CC	R	Local3 Red	37.00a	56.67ab	19.67a
CC	R M	Badillo	41.00cde	64.00cdefg	23.00abc
CC	R M	Local1 RM	37.00a	64.33defg	27.33c
BL	R M	PR1654-1	37.67abc	61.67bcde	24.00abc
BL	R M	PR1654-2	39.00abcd	64.00cdefg	25.00abc
BL	R M	PR1654-3	39.67de	62.00cdef	22.33abc
BL	W	PR1627-10	42.00de	68.33g	26.33bc
BL	W	PR1627-13	41.33de	67.67g	26.33bc
BL	W	PR1627-8	42.33de	67.00fg	24.67abc
CC	W	VERANO	41.33de	66.00defg	24.67abc

CC: cultivar check, BL: breeding line, Be: beige, Bl: black, R: red, RM: red mottled, W: White

Table 3-5. Multiple comparison of means for morphological traits of 23 genotypes

Lines	Bean type	Varieties	Morphological Traits Stem Length (cm)	Pod length (cm)	Pod width (cm)
CC	Be	Local5 Beige	34.59abc	8.94abcd	1.11abcdefg
CC	Bl	Local2 Black	31.74abc	8.89abcd	1.09abcdef
CC	Bl	LORE 234 Local	33.72abc	8.94abcd	1.10abcdef
CC	Bl	MEN2201-64	26.42a	8.75abcd	1.08abcd
BL	Bl	PR1423-110	27.43ab	8.55abc	1.00a
BL	Bl	PR1423-117	27.08a	8.44ab	1.05abc
BL	Bl	PR1423-153	25.30a	8.91abcd	1.04ab
BL	Bl	PR1423-99	26.35a	9.07abcd	1.06abcd
BL	Bl	PR1564-20	27.34ab	8.02a	1.08abcd
BL	Bl	PR1564-3	28.80abc	8.92cd	1.08abcd
BL	Bl	PR1564-53	26.24a	8.49ab	1.13abcdefg
CC	Bl	Salagnac local	38.22bc	9.37bcd	1.17bcdef
CC	Bl	XRAV-40-4	27.83abc	8.55abc	1.06abcd
CC	R	Local3 Red	34.21abc	11.24e	1.31f
CC	R M	Badillo	38.67c	11.77e	1.24ef
CC	R M	Local1 RM	33.29abc	9.08abcd	1.17cdef
BL	R M	PR1654-1	34.69abc	9.86d	1.19defg
BL	R M	PR1654-2	35.88abc	9.83cd	1.21efg
BL	R M	PR1654-3	34.19abc	9.83cd	1.24fg
BL	W	PR1627-10	26.70a	8.07ab	1.13bcdef
BL	W	PR1627-13	26.38a	8.10ab	1.10abcde
BL	W	PR1627-8	26.56a	8.85abcd	1.10abcde
CC	W	VERANO	28.25abc	8.05a	1.13bcdef

CC: cultivar check, BL: breeding line, Be: beige, Bl: black, R: red, RM: red mottled, W: White

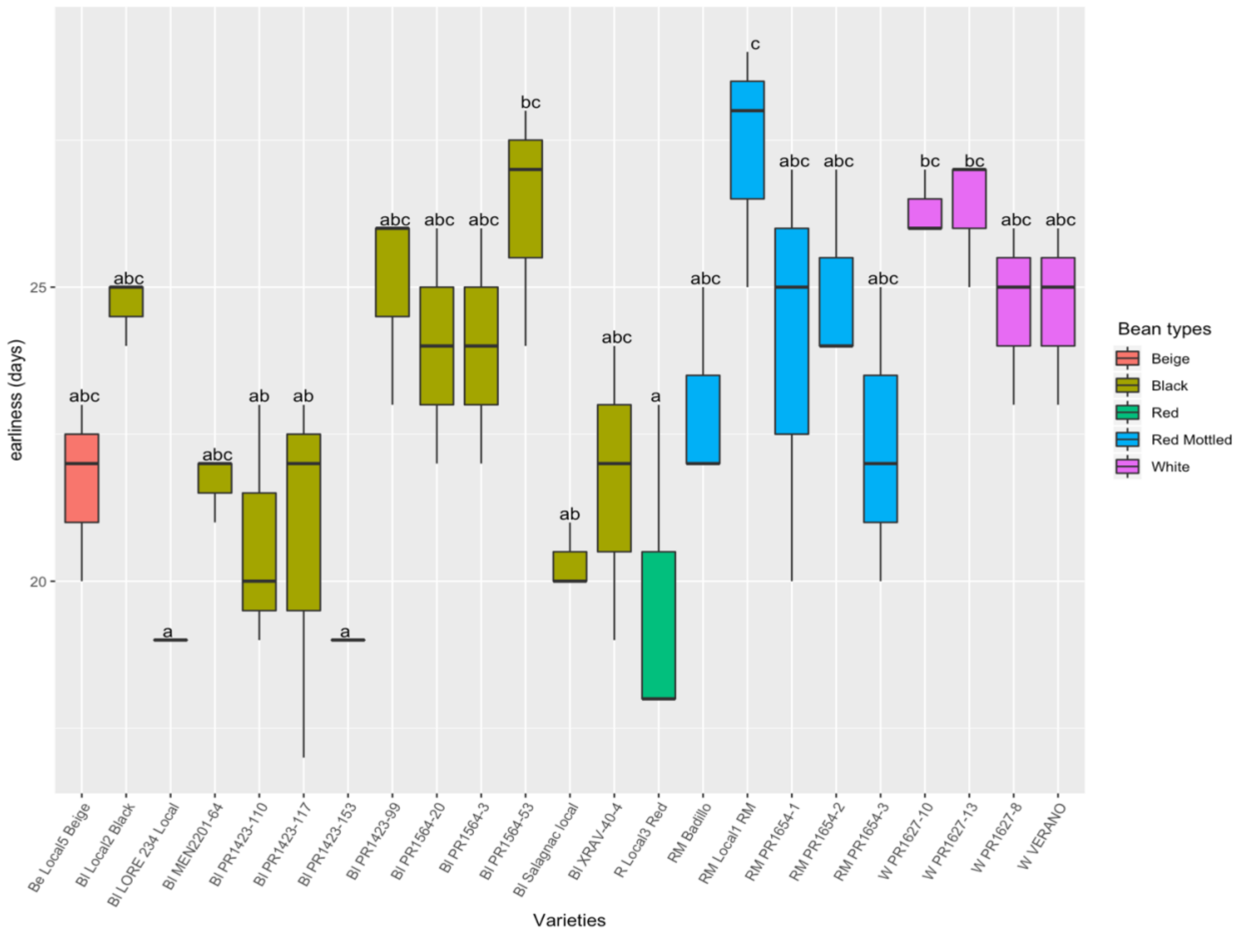

Figure 3-5. Maturity of the genotypes based on difference between days to 50% flowering and to 50% maturity

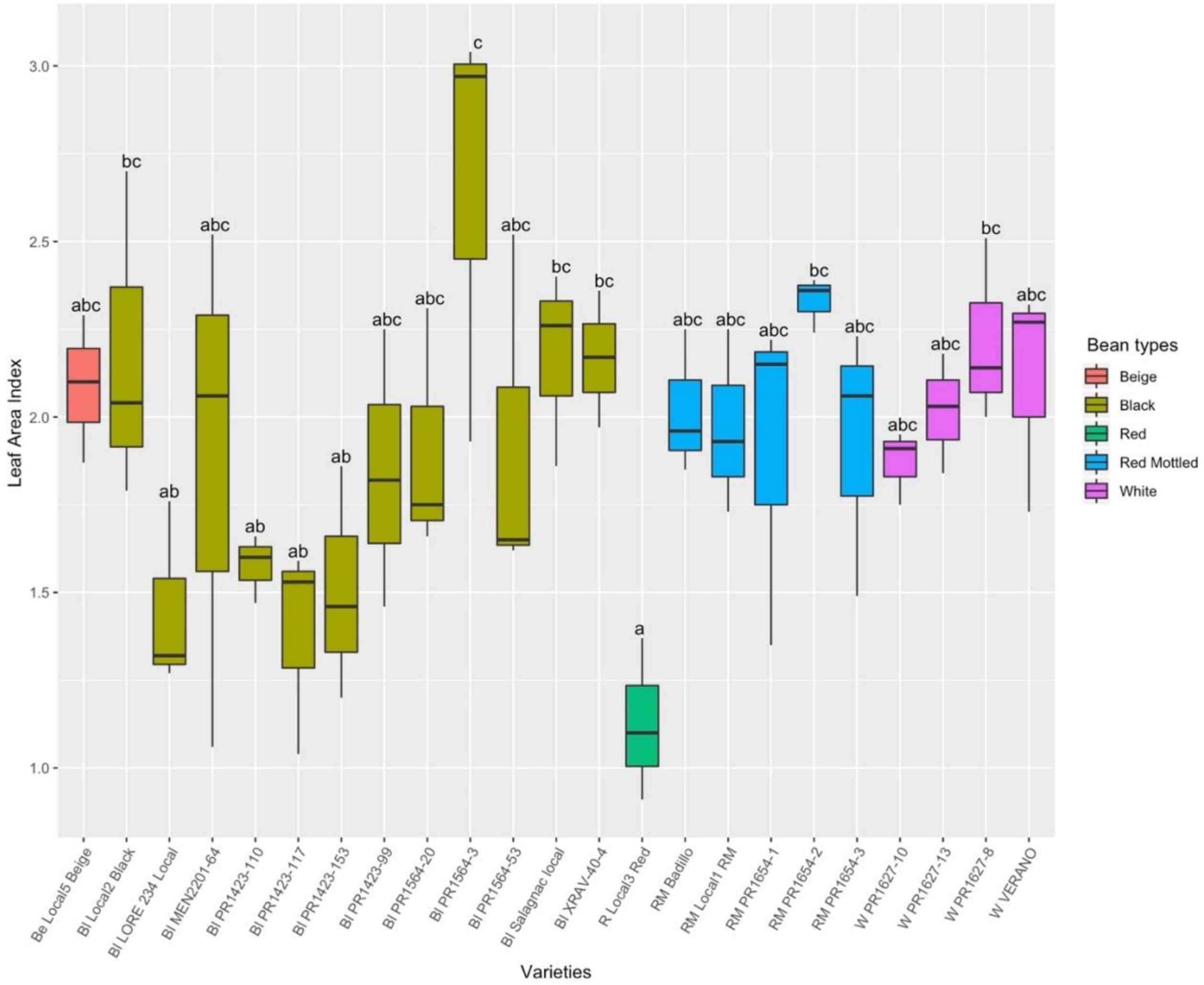

Figure 3-6. Leaf area index recorded for the genotypes

Table 3-6. Qualitative traits measured for the genotypes

Bean type	Lines	growth habit	wing petal color
Beige	Local 5- Beige	Indeterminate type II	white
Black	Local 2- Black	Indeterminate type II	purple
Black	LORE 234 local	Indeterminate type II	purple
Black	MEN2201-64	Indeterminate type II	purple
Black	PR1423-110	Indeterminate type II	purple
Black	PR1423-117	Indeterminate type II	purple
Black	PR1423-153	Indeterminate type II	purple
Black	PR1423-99	Indeterminate type II	purple
Black	PR1564-20	Indeterminate type II	purple
Black	PR1564-3	Indeterminate type II	purple
Black	PR1564-53	Indeterminate type II	purple
Black	Salagnac local	Indeterminate type II	pink
Black	XRAV-40-4	Indeterminate type II	purple
Red	Local 3- Red	Determinate	white
Red Mottled	Badillo	Determinate	white
Red Mottled	Local 1 -Red Mottled	Determinate	white
Red Mottled	PR1654-1	Determinate	pink
Red Mottled	PR1654-2	Determinate	white
Red Mottled	PR1654-3	Determinate	white
White	PR1627-10	Indeterminate type II	white
White	PR1627-13	Indeterminate type II	white
White	PR1627-8	Indeterminate type II	white
White	VERANO	Indeterminate type II	white

Table 3-7. Multiple comparison of means for disease scoring of the bean cultivars

Varieties	Mildew	BGMV	BCMV
Badillo	6.00ef	1.67ab	4.67bcdef
Local 1-Red Mottled	5.67def	2.33abc	6.33fg
Local 2- Black	3.00abc	1.00a	5.00cdefg
Local 3- Red	8.00f	5.33e	6.33fg
Local 5- Beige	3.00abc	3.67bcde	6.00efg
LORE 234 local	3.00abc	5.00de	5.67defg
MEN 2201-64	4.00abcde	2.67abcd	3.00abcdef
PR1423-110	3.33abcd	6.00e	2.33abcd
PR1423-117	3.67abcde	5.33e	2.67abcde
PR1423-153	3.33abcd	6.00e	1.00a
PR1423-99	3.00abc	4.67cde	6.00efg
PR1564-20	2.00a	1.67ab	5.67defg
PR1564-3	2.67ef	1.33ab	6.00efg
PR1564-53	2.00a	1.67ab	5.33cdefg
PR1627-10	2.33a	2.00ab	2.33abcd
PR1627-13	3.67abcde	1.67ab	4.00abcdefg
PR1627-8	2.33a	1.33ab	1.33ab
PR1654-1	5.00bcde	1.33ab	6.67g
PR1654-2	5.33cde	1.33ab	6.00efg
PR1654-3	6.00ef	1.67ab	6.67g
Salagnac local	3.00abc	2.00ab	6.00efg
VERANO	2.67ab	1.00a	2.00abc
XRAV-40-4	2.67ab	2.33abc	5.00cdefg

Table 3-8. Mean value and standard deviation of diseases scored during the field trial

Var	P Mildew	category	BGMV	Category	BCMV	Category
Badillo	6.00±0.0	I	1.67±1.54	R	4.67±1.54	I
Local 1-Red Mottled	5.67±0.15	I	2.33±0.57	R	6.33±0.57	I
Local 2- Black	3.00±1.0	R	1.00±0.0	R	5.00±1.00	I
Local 3- Red	8.00±1.0	S	5.33±0.57	I	6.33±0.57	I
Local 5- Beige	3.00±1.0	R	3.67±1.52	I	6.00±0.00	I
LORE 234 local	3.00±0.0	R	5.00±0.0	I	5.67±1.52	I
MEN 2201-64	4.00±1.0	I	2.67±0.57	R	3.00±1.00	R
PR1423-110	3.33±0.57	R	6.00±1.00	I	2.33±2.30	R
PR1423-117	3.67±0.57	I	5.33±1.54	I	2.67±2.08	R
PR1423-153	3.33±0.57	R	6.00±1.00	I	1.00±0.00	R
PR1423-99	3.00±1.0	R	4.67±0.57	I	6.00±1.00	I
PR1564-20	2.00±0.0	R	1.67±0.57	R	5.67±0.57	I
PR1564-3	2.67±0.57	R	1.33±0.57	R	6.00±0.00	I
PR1564-53	2.00±0.0	R	1.67±1.54	R	5.33±0.57	I
PR1627-10	2.33±0.57	R	2.00±1.00	R	2.33±2.30	R
PR1627-13	3.67±2.0	I	1.67±0.57	R	4.00±1.73	I
PR1627-8	2.33±0.57	R	1.33±1.00	R	1.33±0.57	R
PR1654-1	5.00±0.0	I	1.33±0.57	R	6.67±0.57	S
PR1654-2	5.33±0.57	I	1.33±0.57	R	6.00±1.00	I
PR1654-3	6.00±1.0	I	1.67±0.57	R	6.67±0.57	S
Salagnac local	3.00±1.0	R	2.00±1.00	R	6.00±1.00	I
VERANO	2.67±0.57	R	1.00±0.00	R	2.00±1.73	R
XRAV-40-4	2.67±0.57	R	2.33±0.57	R	5.00±1.73	I

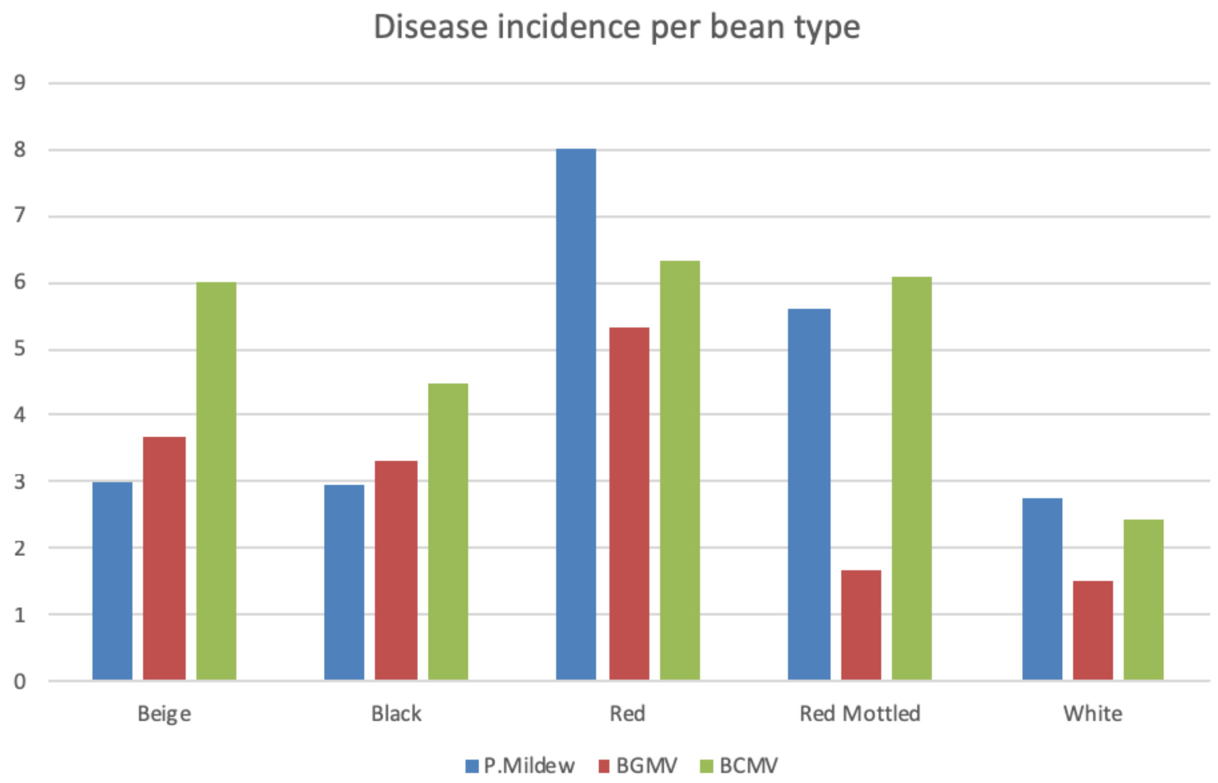

Figure 3-7. Disease incidence according to bean type

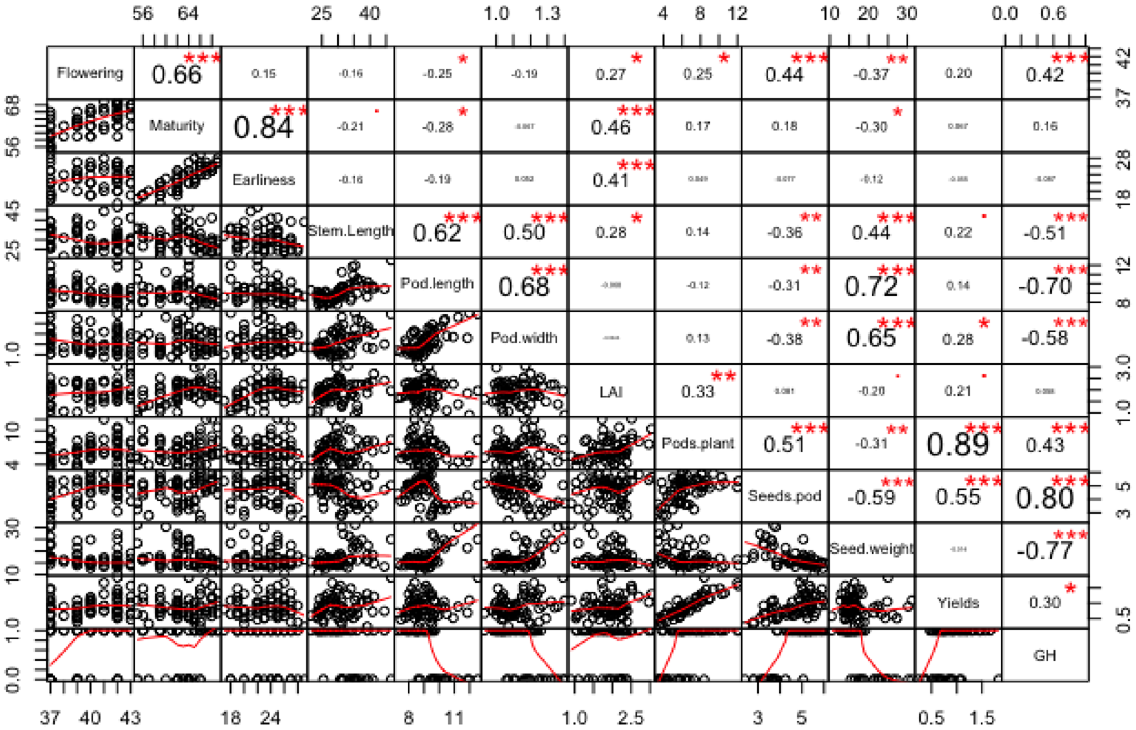

Figure 3-8. Phenotypic correlation

Table 3-9. PCA for the phenotypic traits

	PC1	PC2	PC3	PC4	PC5	PC6
Variation explained (%)	52.99	17.44	12.86	6.52	4.95	2.52
Day to flowering	-0.25	-0.45	-0.11	0.44	0.20	-0.40
Day to maturity	-0.17	-0.58	0.16	0.31	-0.28	0.01
Stem length	0.32	-0.19	-0.20	-0.37	0.37	-0.61
Pod length	0.34	-0.08	-0.31	0.18	0.45	0.26
Pod width	0.35	-0.23	-0.26	0.05	-0.30	0.02
Pods/plants	-0.31	-0.05	-0.42	-0.35	-0.37	-0.13
Seeds/pod	-0.36	0.12	-0.14	0.23	0.47	0.23
Seed weight	0.37	-0.03	-0.23	0.26	-0.12	0.19
Yield (tonnes/ha)	-0.22	0.00	-0.69	-0.01	-0.08	0.21
Leaf Area Index	-0.09	-0.56	0.15	-0.54	0.23	0.48
Growth Habit	-0.39	0.14	-0.06	-0.08	0.15	-0.16

Table 3-9. Continued

	PC7	PC8	PC9	PC10	PC11
Variation explained (%)	1.40	0.72	0.46	0.10	0.06
Day to flowering	-0.11	0.53	-0.08	0.07	-0.16
Day to maturity	0.19	-0.54	0.21	-0.19	0.17
Stem length	-0.03	-0.30	0.23	0.12	0.13
Pod length	0.45	0.02	-0.20	-0.50	-0.01
Pod width	-0.20	-0.22	-0.69	0.28	-0.12
Pods/plants	0.32	0.26	-0.16	-0.12	0.49
Seeds/pod	0.00	-0.24	-0.15	0.48	0.44
Seed weight	-0.54	0.19	0.32	-0.13	0.50
Yield (tonnes/ha)	-0.07	-0.16	0.38	0.10	-0.48
Leaf Area Index	-0.21	0.17	-0.01	0.03	-0.02
Growth Habit	-0.52	-0.27	-0.29	-0.59	-0.02

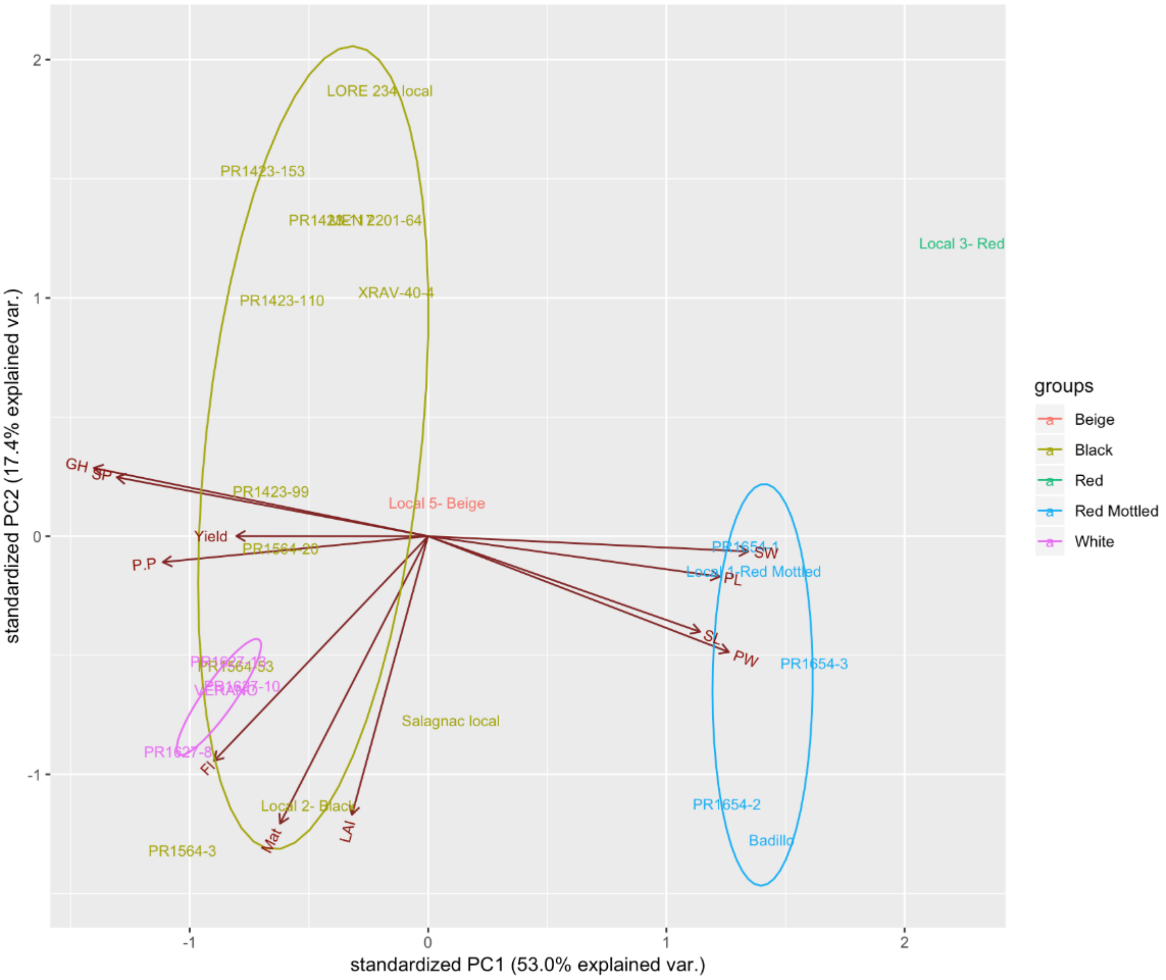

Figure 3-9. PCA plot of the phenotypic traits

CHAPTER 4
GENETIC DIVERSITY AMONG 92 COMMON BEAN VARIETIES COLLECTED ACROSS HAITI

Background

Common bean (*Phaseolus vulgaris* L.) is an important food legume in the world superseded in economic importance only by soybean (*Glycine max* L.) and peanut (*Arachis hypogea* L.). The genus Phaseolus consists of over 30 species (Debouck, 1991), five of which are domesticated; P. *acutifolious* A. Gray (tepary bean), P. *coccineus* L. (runner bean), P. *lunatus* L. (lima bean), P. *polyanthus* Greenman (year-long bean), and P. *vulgaris* L. (common bean or snap bean) (Debouck, 1999). Among these, common bean is most cultivated across the globe and forms an essential source of nutrition and income in Latin America and Eastern and Southern Africa (Broughton et al., 2003). Common bean is postulated to have independently originated from two centers of diversity, which led to the formation of Mesoamerican and Andean gene pools (Blair et al., 2009). The delineation between the two gene pools is supported by distinct molecular signatures observed in phaseolin seed proteins (Gepts et al., 1986), allozymes (Singh et al. 1991), morphological traits (Singh et al., 1991), and DNA markers (Becerra et al., 1994). The gene pools are further divided into six races according to morphological criteria and agro-ecological adaptation, three each for Mesoamerican (Durango, Jalisco, and Mesoamerica) and Andean (Chile, Nueva Granada, and Peru) (Blair et al., 2009).

Knowledge and understanding of the genetic diversity among bean germplasm collection are essential for conservation efforts, as well as for broadening of the genetic base of varieties. Although many bean varieties are grown in Haiti, little information is available on their genetic structure, thus hindering germplasm improvement efforts.

Besides, most growers do not identify varieties by name, instead of by market class, thus making it difficult to determine the diversity of cultivated common bean in the country. Characterization of germplasm using DNA molecular markers provides quantitative estimates of genetic diversity (Manifesto et al., 2001). A diverse number of markers are applied in assessing genetic diversity in beans including amplified fragment length polymorphisms, simple sequence repeats (SSR) and resistance gene analogs (Blair et al., 2013, Grisi et al., 2007; Yu et al., 2000) and single nucleotide polymorphism (SNPs) (Campa et al, 2018; Valdisser et al, 2017).

DNA sequencing has become feasible with the advancement of next-generation sequencing (NGS) technologies. NGS technologies such as genotyping-by-sequencing (GBS) have made DNA sequencing faster and cost-effective. The GBS method has been extensively used for species with high diversity and large genome (Campa et al., 2018). GBS method generates thousands of SNP markers across the genome and has been used extensively in common bean for genome-wide association study (GWAS), high-density linkage map construction and diversity study (Hart et al., 2015; Katuuramu et al., 2018, Bhakta et al., 2015, Campa et al, 2018).

The goal of the current study was to determine the genetic diversity among 92 bean genotypes collected from different agro-ecological zones across Haiti using GBS technology.

Materials and Methods

Plant Materials

Seeds of ninety-two lines of beans were collected from different agro-ecological zones of Haiti. Twenty-three of these lines were obtained from the USAID-AREA and the Legume Innovation Lab programs. In addition, four control genotypes that are commonly used for discriminating Mesoamerican and Andean gene pools in previous diversity studies were included (Asfaw et al. 2009; Blair et al. 2009, 2016). The controls are G4494 (Calima) and G19833 (Chaucha Chuga) for the Andean gene pool and Dorado (DOR 364) and G5773 (ICA Pijao) for the Mesoamerican gene pool (Blair and Lorigados, 2016).

Seed Germination and DNA Extraction

For each genotype, twenty-five seeds were germinated in cells filled with Jolly Gardener PRO-LINE C/B Growing Mix medium in the greenhouse (22°- 32°C) at the University of Florida- Tropical Research and Education Center, Homestead, Florida. At the two-leaf stage, one leaf of about 2 cm was collected from 20 individuals of each genotype and immediately frozen in liquid nitrogen. DNA was extracted using a modified protocol that combined a differential centrifugation step from the nuclear fraction protocol (Bhakta et al., 2015), and the Flavorgen Biotech DNA isolation Kit. 100 mg of ground tissue was placed in 2 ml Eppendorf tube using 1500 ul of sample resuspension buffer (SRB) with the inclusion of 0.5 % Triton X-100 and 1% of beta-mercaptoethanol. The tubes were spun at 1500 rpm for 10 minutes. Subsequently, the supernatant was discarded, and the pellet was used for the DNA extraction using the Flavorgen kit. The use of differential step with the inclusion of Triton X-100 detergent allowed to remove the cytoplasmic DNA and obtain high quality of nuclear DNA, which is required for sequencing. The detergent dissolves the plastid, and mitochondrial membranes, therefore, allow the release of cytoplasmic DNA in the buffer solution (Bhakta et al., 2015). The DNA quality was evaluated by using 0.8% agarose gel, and the concentration was assessed using Fisher Nanodrop One and Qubit 4 fluorometer.

Genotyping by Sequencing

Genotyping by sequencing was conducted according to Schröder et al 2016, at Georgia Genomics and Bioinformatics Core at the University of Georgia using double digestion with MseI and TaqαI restriction enzymes. This method was used because it has been shown to improve the quality and the coverage of GBS data in common bean (Schröder et al., 2016). The GBS library was prepared by ligating the digested DNA to unique nucleotide adapters (barcodes) followed by PCR with flow-cell attachment site tagged primer. Illumina NextSeq 150x High output Flow Cell was used to perform the sequencing. Demultiplexing with quality filtering was conducted by using Stacks. The sequencing reads were aligned to the P. Vulgaris L. reference genome sequence using Burrow Wheelers Alignment (BWA) tools. The reference-based pipeline in Stacks was used for the extraction of single nucleotide polymorphism (SNP). Data filtration was performed in Tassel 5.2.52 by considering missing data inferior to 70%, and minor allele frequency (MAF>0.01).

Data Analysis

The population structure analysis was conducted with Structure v2.3.4. The Structure parameters used were admixture model with independent allele frequencies, a burn-in period of 1000 and 5000 Markov Chain Monte Carlo (MCMC) iterations with 20 replications for each hypothetical number of subpopulations (k) between 1 and 5. The optimum K value was calculated according to Evanno et al. (2005) using Structure Harvester. A new burn-in period of 10000 and 30000 MCMC iterations were conducted for the optimum K value to assign accessions to subpopulations (Campa et al., 2018). Cluster analysis and principal component analysis (PCA) was conducted by using Darwin 6.0.021.

Results

Genotyping

The goal of this project was to assess the genetic diversity of 92 common bean lines from Haiti using Next Generation Sequencing technology. Genotyping was conducted using Illumina NextSeq. Sequencing of the GBS library yielded approximately 454,854,048 reads, while the Q30 value exceeded 80%. A total of 27,823 SNPS were identified. After filtering for missing data and minor allele frequency, a total of 1,115 SNPs distributed across the eleven chromosomes was selected. The number of SNPs per chromosome ranged from 62 on chromosome Pv6 to 169 on chromosome Pv11, with an average of 98 markers/ chromosome.

Population Structure

The Structure v2.3.4 software was used for testing a hypothetical number of subpopulations from one to five. The ΔK value designated an optimal amount of two subpopulations (Fig 4-1 and Fig 4-2). The two main groups identified (Fig 4-3) included a group of 8 lines closely related to the controls G4494 and G19833 from the Andean gene pool and a group of 84 lines closely related to Mesoamerican controls (DOR 364 and G5773). However, 12 lines showed some level of admixture between the two gene pools.

Phylogenetic Tree

A dendrogram was constructed using UPGMA in Darwin using 1,115 SNPs. Figure 4-4 showed the phylogenetic tree obtained. From this tree, two main groups were obtained, a small group that included 2 Andean check cultivars G4494 and G19833 and eight other lines, and a large group that included 2 Mesoamerican controls (DOR 364 and G5773, in addition to 84 other lines which are predominantly small-seeded black beans. A certain level of admixture was found in 2 lines in the Andean and ten lines in the Mesoamerican subpopulation.

Principal Component Analysis

A two-dimensional plot obtained in the principal component analysis (PCA) is presented in Figure 4-5. The first component PC1 accounted for 10.7% of the variances and distinguished the two main groups, Andean and Mesoamerican, as two separate clusters. The lines that showed introgression were identified through Structure and are mainly clustered in the intersection between the two main groups. On the other hand, the second PC accounted for 2.49% of the variance and revealed more diversity within the Mesoamerican than within the Andean gene pool. According to the PCA in Figure 4-6 which displayed the genotypes based on market type, the Mesoamerican group contained diverse bean types, including small-seeded black beans which are prevalently grown and consumed in Haiti, yellow bean, white, and red mottled whereas the Andean group was composed mostly of large-seeded red and red mottled beans.

Discussion

This project aimed to determine the genetic diversity among a collection of common bean accessions in Haiti and is the first of a kind to use genome-wide based molecular markers derived through GBS.

The accessions included in the current study are a good representation of the different agro-ecological regions in the country since they were collected from small-scale farmers across the country who typically save seed for subsequent growing seasons. The bean pool also contained lines from different breeding programs such as ORE (Organization for Rehabilitation of the Environment), University of Puerto Rico and UF-Haiti AREA legume breeding program, University of Nebraska, USDA-ARS, University Zamorano and National Seed Service (SNS) Haiti.

Structure, cluster, and principal component analysis (PCA) revealed the presence of the two main gene pools, Andean and Mesoamerican, in the Haitian bean collection. The separation between the two groups was confirmed by the presence of the Andean controls G4494 and G19833 in the Andean subgroup (Fig 4-4), and the Mesoamerican controls (DOR 364 and G5773) in the Mesoamerican subpopulation. Separation of the two gene pools has been observed in bean collections from the Carribean using RAPD and phaseolin analysis markers (Durán et al. 2005, Castiñeiras et al., 1991, Lioi et al., 1990).

Most of the lines (87%) collected clustered within the Mesoamerican gene pool. This result indicates that growers may prefer accessions of the Mesoamerican gene pool (black beans, yellow beans, white beans, and red mottled) due to higher yields. Additionally, this gene pool is typically well-adapted to high-temperature conditions in Haiti (Blair and Lorigados, 2016). In the current study, red mottled bean types clustered in both gene pools. However, the majority of these accessions were genetically similar to the Mesoamerican controls. These results confirmed previous findings that found a predominance of Mesoamerican-type red mottled lines in Haiti, the Dominican Republic, and Cuba (Blair and Lorigados, 2016; Durán et al., 2005).

Although, the population structure analysis revealed two main subpopulations in the bean collection evaluated, 12 cultivars (about 13%) showed some level of introgression between the two gene pools. The level of introgression found between the two gene pools in Haiti is lower than that reported in other countries including China, Italy, Ethiopia, Kenya, Spain, Portugal, Rwanda, which represent secondary centers of diversity for common beans (Asfaw et al, 2009; Blair et al, 2010, Masi et al.,2009; Martins et al, 2006; Rodiño et al, 2003; Santalla et al, 2010; Sicard et al, 2005; Zhang et al, 2008). Previous molecular characterization studies of common bean landraces and cultivars from the Caribbean revealed possible introgression between the two gene pools (Durán et al., 2005). Similar admixture levels were found among common bean accessions from Cuba (Blair and Lorigados, 2016) and Brazil (Blair et al., 2013; Burle et al., 2010). The admixture observed between the two gene pools might be expected due to inter-gene pool breeding efforts that primarily utilize the Mesoamerican genetic background to improve specific characteristics of Andean bean lines (Beaver, 1999; Durán et al., 2005). Besides Haiti, an active breeding program in the tropics, particularly in the highlands of eastern and southern Africa have resulted in increased admixture between the gene pools (Blair and Obrigado, 2016; Díaz et al. 2011; Blair et al., 2010; Díaz and Blair, 2006). The level of inter-gene pool introgression is lower in regions of common bean domestication, including the Mesoamerican region of Central America and Mexico, and the Andes mountains of South America (Blair and Obrigados, 2016; Avila et al., 2012; Blair et al., 2011).

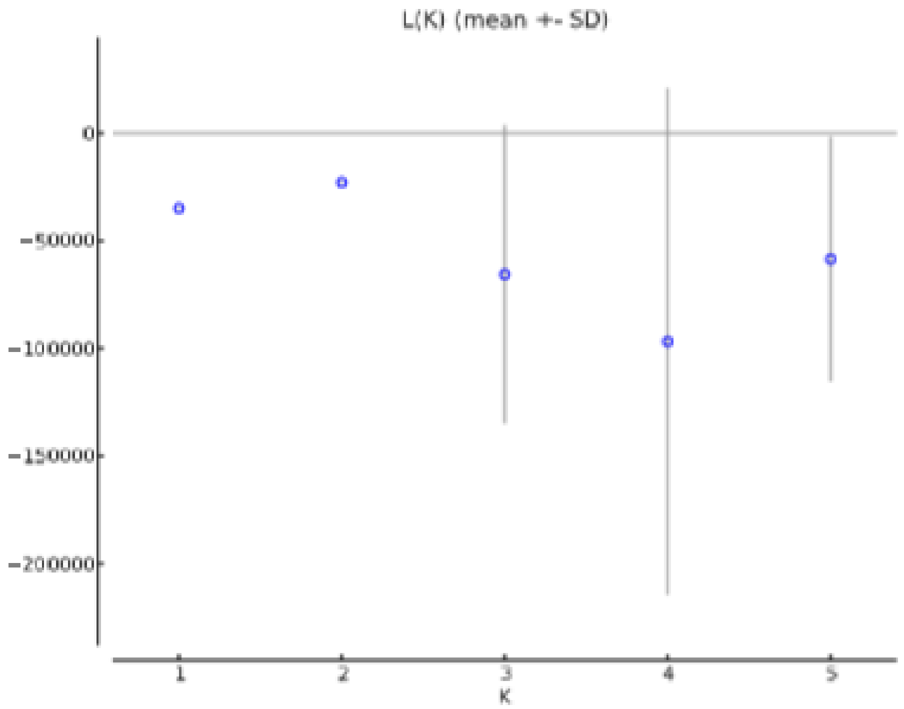

Figure 4-1. Mean L(K) (±SD) over 20 runs for each K value

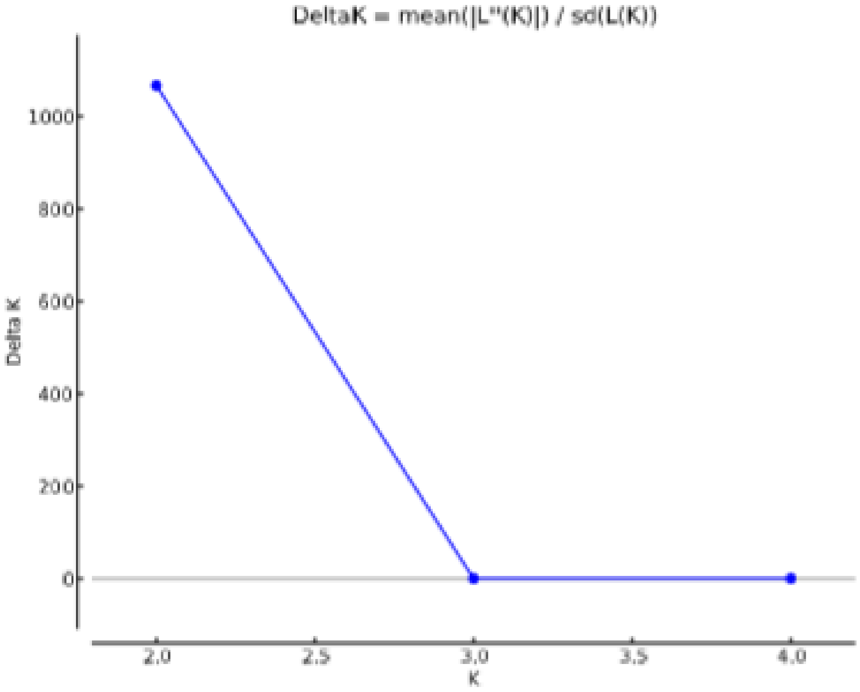

Figure 4-2. Plot of Δ

Figure 4-3. Population structure inferred by Bayesian approach based on 1115 SNPs for k=2, Mesoamerican (red), Andean (green)

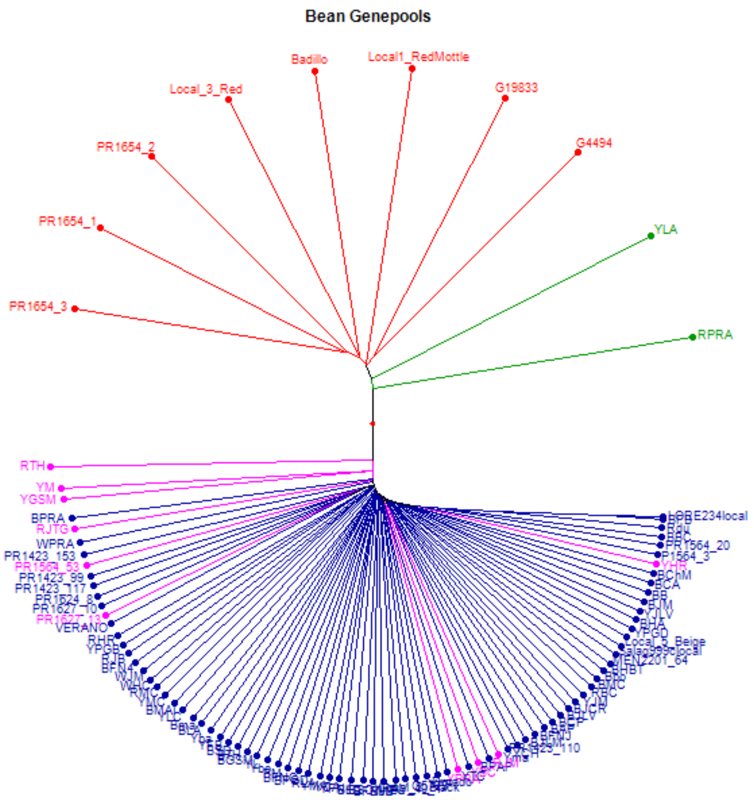

Figure 4-4. Phylogenetic tree obtained for 96 lines of common bean and 1115 SNPs

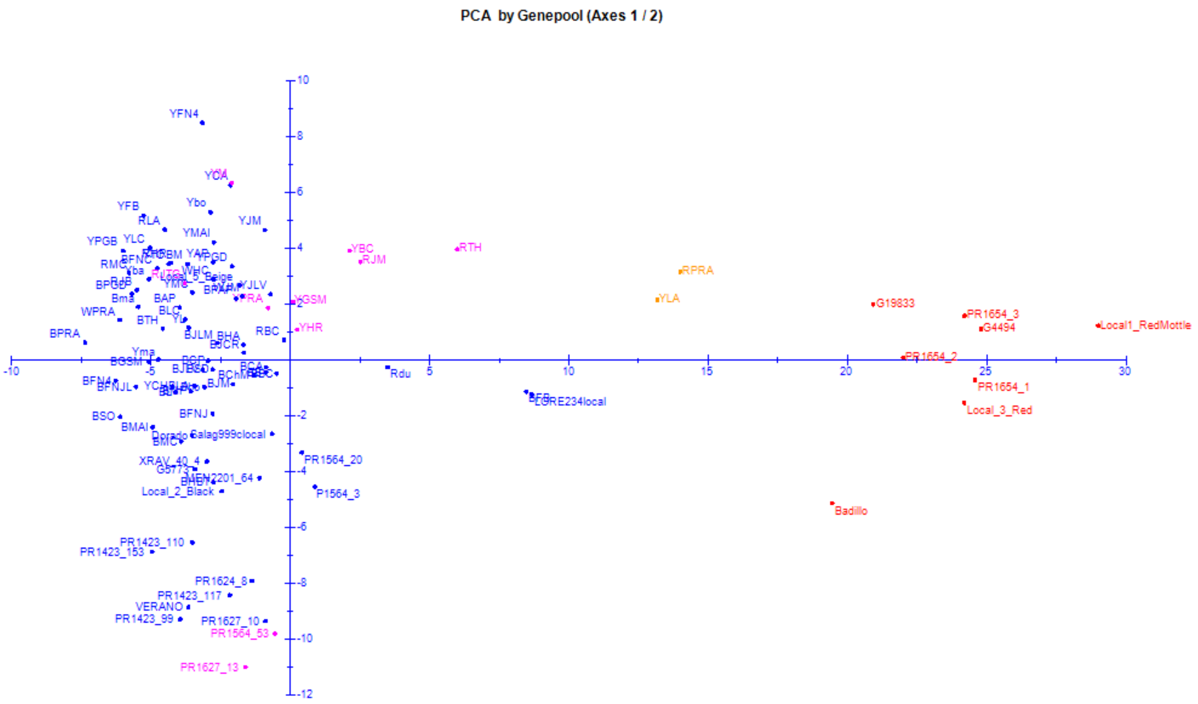

Figure 4-5. Two-dimension plot obtained from principal component analysis (PCA) for 96 lines of common bean and 1115 SNPs based on gene pool

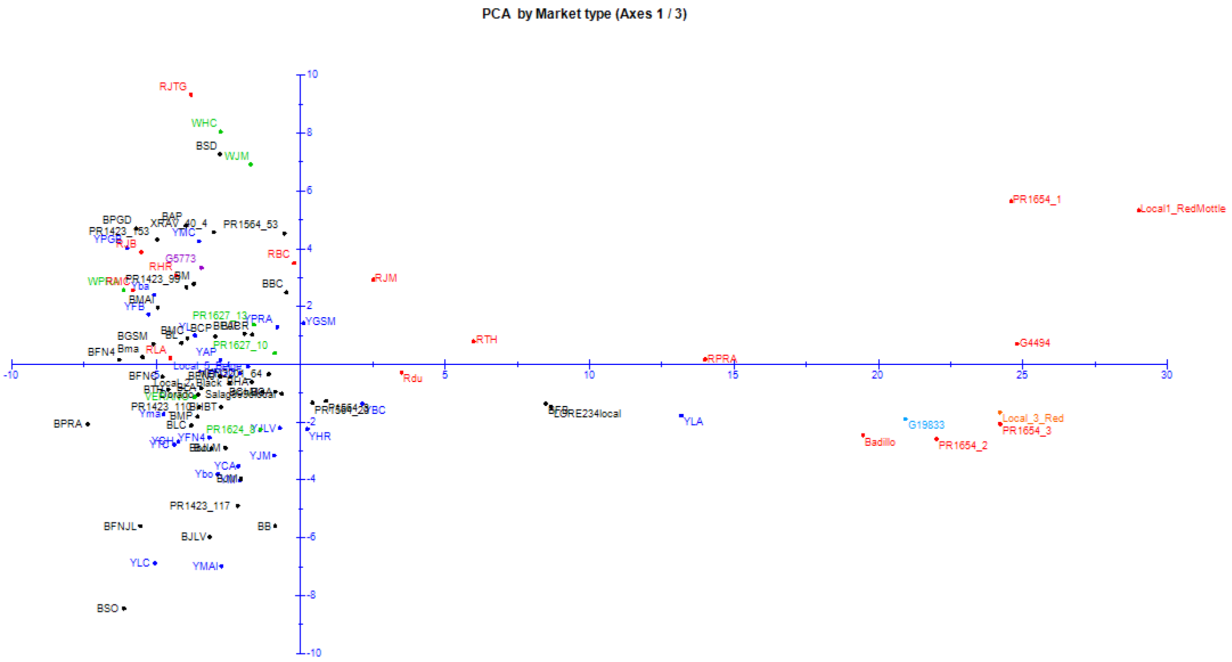

Figure 4-6. Two-dimension plot obtained from principal component analysis (PCA) for 96 lines of common bean and 1115 SNPs based on bean market type

CHAPTER 5
SUMMARY AND CONCLUSIONS

In the current study we evaluated the agronomic performance of 13 elite common bean-breeding lines currently under development by the UF-AREA and the Legume Innovation Lab programs in Haiti. The results reported in this study showed a significant difference among the genotypes for most of the traits measured. The PR1627-8 (white bean) outperformed the other breeding lines in yield and disease resistance; therefore, it is a candidate for release to growers. Furthermore, the cultivars checks, including LORE 234 local, and Local 3 red could be used as a source of earliness for breeders. Based on phenotypic relationships, the number of pods per plant and number of seeds per pod may be useful traits for indirect selection for seed yield in common bean. However, the data used were only for one season and one location; therefore, further trials are necessary to validate the results. Besides, this current project has some limitations that need to be considered when interpreting the results reported here. This research was conducted under conditions typical for Haitian farmers (agriculture with low input) with the goal to select varieties that can thrive under different biotic and abiotic stress with minimal input. The field trial was rainfed with no fertilizer and pesticide applications. Furthermore, the varieties differed in their response to the diseases assessed (powdery mildew, BCMV, and BGMV). The growth habit of the different gene pools may also affect the yield of the genotypes. Nevertheless, to reduce the effect of some of the cofounding factors, disease incidence, as well as growth habit, were included in the ANOVA model for yield.

The genetic diversity was also assessed among a collection of 92 common bean lines from Haiti, which included landraces, breeding lines, and released cultivars. The GBS revealed 27,823 SNPs, among which 1,115 were used for the diversity analysis after filtering for missing data and minor allele frequency. Structure, cluster, and principal component analyses revealed the presence of the two main gene pools in the bean population in Haiti. From these two subpopulations, the Mesoamerican gene pool was predominant with about 87% of the accessions collected, thus reflect the preference of this bean type among Haiti consumers. However, 12 lines showed some level of introgression between the two main gene pools. The principal component analysis revealed more extensive genetic diversity within the Mesoamerican than within the Andean gene pool in the accession collection.

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BIOGRAPHICAL SKETCH

Riphine Mainviel was born in Petit-Goâve, Haiti. She earned a Bachelor of Science degree in Agriculture with a major in natural resources and environment at the Faculty of Agronomy and Veterinary Medicine (FAMV) of the State University of Haiti in 2014. Upon graduation, she worked for the Ministry of Agriculture of Haiti in the Mitigation and Natural Disaster Program from 2015 to 2017. She was granted a scholarship in 2017 from UF-IFAS AREA project to pursue her master’s degree in Horticultural Sciences Department, University of Florida. She was working with Dr. Geoffrey Meru at TREC/ Homestead along with Dr. C. Eduardo Vallejos and Dr. Raphael Colbert as her committee members. Her primary research involved analyzing agronomic performance and genetic diversity of common bean varieties in Haiti.